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A BACTERIAL STRIPE DISEASE OF PROSO MILLET[1]

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INTRODUCTION

Proso or broomcorn millet (*Panicum miliaceum* L.) is the common millet of the Old World (*4*).[2] Especially in India, Russia, and China it is an important crop for human food. From these countries, principally Russia, a large number of different varieties have been introduced into the United States and tested experimentally, and about a dozen varieties are now grown to some extent in the northern Great Plains States. Although proso is easily injured by frost and grows well only with moderately high temperatures, the length of time from seeding to maturity—50 to 90 days—is comparatively short and makes this grain of value as a supplementary or catch crop. It is grown to a considerable extent in the drier parts of Europe and Asia, and in our Great Plains States sometimes produces a larger yield in dry seasons than other small grains.

The leaves and stems of proso are covered with hairs which make it undesirable for hay. The different varieties grow from 1½ to 3 feet high and are otherwise distinguished by type of panicle, color of seed and chaff, and time of maturity. Proso (*Panicum miliaceum* L.) differs from the more common foxtail millets (*Chaetochloa italica* (L.) Scrib.) in having more open branching panicles somewhat resembling those of the oat inflorescence. The seed is larger and makes better feed. While the foxtail millets are grown especially for hay, the prosos are grown for the seed which is fed to hogs, poultry, and other live stock.

Early Fortune is one of the leading varieties of proso and has been grown in the United States for many years. It grows only 1½ to 2 feet high and matures very early. Some of the other varieties mentioned in this paper (Black Voronezh, Hansen's White Siberian, Tambov, Turghai) have been introduced from Russia since 1897 and are grown to some extent at the experiment stations of the Mississippi Valley.

DESCRIPTION OF THE DISEASE

The first known observation of a bacterial disease on proso was made at the South Dakota Agricultural Experiment Station, at Brookings, in August, 1917, by Dr. A. G. Johnson. He described the lesions on Hansen's White Siberian as characteristic water-soaked to brown stripes

[1] Accepted for publication May 2, 1923. This work was done in cooperation with the Office of Cereal Investigations, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] Reference is made by number (italic) to "Literature cited," p. 159.

on leaves, sheaths, and culms. The streaks were shiny, and the interiors of some stems showed an abundance of gummy exudate. In some plants the main culms had been killed and were brown and dry, and only spindling side culms were living. Other plants showed that the upper portion, including the head, had been killed. Specimens of diseased plants were collected, but no further work was done.

In July, 1920, the writer found badly diseased plants of Early Fortune (S. D. No. 98) in the plots at Hill Farm, Madison, Wis. (Pl. 1, 2, 3, A). The seed for the plot had come from the South Dakota station, and the lesions were like those described earlier by Doctor Johnson. The plants were about 18 inches high and just beginning to head. Narrow, brown, water-soaked streaks extended from the blades of the leaves down onto the sheaths, and were also present on the culms. Where many streaks coalesced on a leaf the tissue was brown and translucent, and there was evidence of abundant exudate in the form of thin, white scales along the streaks. Similar lesions also occurred on the peduncles and pedicles of the panicle. In most cases the infection was not severe enough to kill the plants, but individual leaves were partly or entirely browned. In some instances the whole top of the plant was killed, the tissue becoming soft and brown, especially where partly inclosed and protected by lower leaves and sheaths. In such cases new shoots were coming out at the base.

The groups and varieties of millet grown at Madison in 1920 are shown below:

Barnyard millet (*Echinochloa crusgalli edulis*):
Japanese millet..... S. P. I. No. — C. I. No. — S. D. No. 101

Proso millet (*Panicum miliaceum* L.):
Black Voronezh..... S. P. I. No. 2795 C. I. No. 16 S. D. No. 9
Early Fortune..... C. I. No. 23 S. D. No. 9
Hansen's White Siberian..... C. I. No. 179 S. D. No. 99
Tambov..... S. P. I. No. 2794 C. I. No. 13 S. D. No. 8
Turghai..... S. P. I. No. 10625 C. I. No. 31 S. D. No. 9

Foxtail millet (*Chaetochloa italica* (L.) Scribn.):
Ainu.....
Common..... S. D. No. 3
German (Golden)..... S. D. No. 101
Kursk..... S. D. No. 7
Kursk (Shelley)..... S. D. No. 34

These millets were grown in a series of adjoining, duplicate rows in the following order: Japanese millet, Black Voronezh, Hansen's White Siberian, Ainu, Tambov, Early Fortune, Turghai, German (Golden) Kursk (Shelley), Kursk, Common.

The disease developed only on varieties of proso. Early Fortune showed abundant infection. A few scattered lesions appeared on Tambov and Turghai which grew on either side of Early Fortune. Traces of the disease also appeared on Hansen's White Siberian. The varieties of foxtail and barnyard millets showed no signs of infection, although two varieties of foxtail millet were growing beside infected varieties of proso.

Seed of Early Fortune from Madison, Wis., was sown at Tuxedo, Md., in 1921. The season was hot and dry, and only scattered lesions appeared on the plants. Seed from this 1921 plot was again sown in a different field at Tuxedo, Md., in May, 1922. During May and early June there were abundant and almost daily rains for two weeks. Rows of Hansen's White Siberian and of Tambov were sown in the same plot. All three varieties showed abundant infection with the bacterial disease. Every plant of Early Fortune showed more or less infection, and in about 10 per cent

of the plants the growing point and upper unfolding leaves were killed. The killed portions of the expanded leaves were dry and brown, while the killed, inclosed, young leaves were soft, brown, and disintegrating. Lesions extending down the sheaths and stem to the root produced a browning at the crown where the plants readily broke away from the root. There were abundant separate lesions on blades and sheaths, and many of the leaves looked brown and ragged where the tissue had split at the lesions. The other two varieties showed abundant infection, but not so much as Early Fortune. This plot was practically destroyed by the disease.

ISOLATION AND INOCULATION EXPERIMENTS

Microscopic examination of the free-hand sections through leaf blade and sheath showed that there were large numbers of bacteria in the lesions.

Isolations from fresh material were made in two ways: (1) Scales of exudate were scraped off into +15 broth and plates poured from the broth; (2) small pieces of leaf tissue were dipped into 95 per cent alcohol for a few seconds, then placed in mercuric chlorid solution (1:1000) for one to two minutes, washed through three sterile water blanks, crushed and transferred to sterile broth from which the plates were poured. Twelve isolations were made at different times and all gave practically pure cultures of a white organism which, when used for inoculation, produced typical lesions of the disease on proso. Transfers from these isolations were labeled cultures I, II, III, IV, V, etc., and cultures I, II, and V were used for cultural and morphological studies.

Inoculations were made by spraying plants in the greenhouse with water suspensions of the organism made from 2-day-old to 4-day-old agar plant cultures. Sprayed plants were held in damp chambers for from two to four days. Controls were sprayed with sterile water and held under the same conditions as inoculated plants.

In three to four days the lesions appear on the inoculated leaves as water-soaked streaks a millimeter in diameter and from one to several centimeters in length (Pl. 3, B). The lesions are sometimes along the margins and sometimes through the leaf blade between the veins. Lesions may extend from the blade down onto the sheath. Some plants rotted at the surface of the soil where the inoculum had run down and collected. In one inoculation with culture I, where there were a great many infections, the leaves turned yellow or dried up. Controls under identical conditions kept a healthy green color and showed no signs of infection. Reisolations from these lesions gave characteristic white colonies which again produced narrow, water-soaked lines 3 to 4 inches long on the leaf blades.

Out of 15 inoculations of Early Fortune, 11 produced good typical lesions of the bacterial disease, while 2 were unsuccessful because the damp chambers were not tight and the plants could not be kept moist. The other two inoculations were made with isolation No. IV, which never produced any lesions and was discarded as not being the organism to which the disease is due.

Six inoculations were made on sorghum (African, Orange, and an unidentified variety) with cultures I, II, and V. No lesions appeared on any of the plants, although proso plants inoculated at the same time and kept in the same damp chambers showed abundant infection. Acme

broom corn, C. I. No. 243, was sprayed twice with water suspensions of culture I without producing any lesions.

Feterita and Dwarf kafir were both sprayed with cultures I and V, and no lesions appeared.

A Feterita-Milo hybrid was twice sprayed with culture I, without any evidence of infection in one experiment, but in the other, after three weeks, irregular reddish-brown streaks appeared on the margin and toward the center of one leaf blade. Although this plant was resprayed with the proso organism, no new lesions appeared and the original lesions did not spread. Typical colonies of the proso organism were not isolated from this plant and there was considerable doubt as to whether the infection was due to this organism.

Although no lesions appeared on inoculated plants other than proso, typical lesions always appeared on proso plants sprayed with isolations of the organism at the same time and kept in the same damp chambers. The only exceptions were the two cases mentioned above where the damp chambers failed to keep the plants moist.

CULTURAL AND MORPHOLOGICAL CHARACTERS

The organism producing the disease of proso described above is a short rod with rounded ends, arranged singly or in pairs. Occasionally chains occur. Two-day cultures on potato cylinders (stained with gentian violet) vary from 3.1μ to 1.53μ in length and 0.9μ to 0.45μ wide, with an average of 2.08μ by 0.67μ. From two-day +15 agar cultures, stained with carbol fuchsin, they vary from 2.3μ to 1.1μ long by 0.9μ to 0.5μ wide, with an average of 1.23μ by 0.67μ. In one-day cultures on potato cylinders stained with gentian violet, the range is 2.35μ to 1.17μ by 0.9μ to 0.45μ with an average of 1.67μ by 0.73μ.

There are no spores, endospores, zoögloea, or pseudozoögloea. Capsules are present on beef peptone agar and other media, but do not stain readily with Ribbert's capsule stain or other stains (Pl. 4, G).

Usually there is one polar flagellum, but occasionally there appear to be two or three. Flagella were stained with Casares Gil's stain (Pl. 4, H, I).

At 33° C. cultures of the proso organism grown for nine days on beef-peptone agar and stained with carbol fuchsin show peculiar club-shaped growths with one end more or less enlarged and the other a short or long tail-like extension. These involution forms were observed only at high temperature (Pl. 4, F).

STAINING REACTIONS.—All strains of the proso organism stain readily with carbol fuchsin and gentian violet but only lightly with methylene blue. There is definite polar staining (Pl. 4, I), especially with carbol fuchsin, which does not stain quite so heavily as gentian violet. The strains are Gram negative and not acid fast.

NUTRIENT BROTH.—In +15 (Fuller's scale) beef-peptone bouillon there is moderate clouding in 24 hours and heavy in 48 hours. A thin pellicle forms over the surface which readily breaks up and falls in thin flakes. Often when broth cultures are a day old and the clouding is still light, a very thin pellicle forms and from this the clouding extends down into the tube in fine strands. Sometimes as cultures grow older the pellicle is heavier in the center, which hangs down into the broth. After about two months' time the inoculated tubes are a deeper color than the controls—a little darker than Ridgway's Buckthorn Brown.

There is a distinct odor of decay. When broth cultures have been inclosed in a case or chamber for some days the odor is strong and disagreeable. Only a small quantity of sediment is formed at the bottom of the tube which on being shaken appears to be more or less viscid but breaks up rather readily to form a part of the clouding.

AGAR SLANT.—Growth on +15 (P_H 6.8) beef-peptone agar is moderate, filiform, slightly raised, shining, smooth, translucent, white, sometimes iridescent, of butyrous consistency to somewhat viscid. There is a strong odor of protein decomposition. The medium is unchanged.

AGAR COLONIES.—On +15 beef-peptone agar colonies grow slowly at room temperature, measuring 1 to 1.5 mm. in 48 hours, and 1 to 5 mm. in 4 days. Colonies are round, white, slightly iridescent, smooth, shining, raised. The margin is entire at first but after 3 to 4 days wedge-shaped growths appear (Pl. 4, C) around the outer edge which gives an undulate margin. Internally, the colonies are finely granular but the wedge-shaped growths have fine markings similar to those of the wheat and barley organisms. There is a strong odor similar to that of *B. coli*. The center of the colony is denser and quite definitely marked off from the rather broad margin. Growth on potato agar is similar.

GELATIN COLONIES.—Growth is slow. Surface colonies are circular, raised, with margins entire, and internal structure granular (Pl. 4, A). Embedded colonies are irregularly lobed (Pl. 4, B). Liquefaction is saucer-shaped, and takes place slowly at room temperature. In the ice box there is no evident liquefaction. The colonies grow very slowly and the media dries as fast as any liquefaction takes place. The only evidence of liquefaction in the ice box is the fact that surface colonies grow in saucer-shaped depressions. In one set of plates the surface colonies after 12 days showed cup-shaped depressions in the centers of the colonies (Pl. 4, D, E).

GELATIN STABS.—Growth is best at the top with slight filiform growth along line of puncture. Liquefaction is crateriform and very slow in the ice box. Tubes held in the ice box for a year were entirely liquefied. One set of tubes were kept at room temperature. The controls did not liquefy, but at the end of two months the inoculated tubes were about half liquid.

POTATO CYLINDERS.—Growth is moderate in amount, filiform, flat, glistening, smooth, butyrous, light cinnamon-buff to tawny olive (Ridgway), with a penetrating odor of decay. The cylinders are grayed. There is moderate diastasic action on starch.

TEMPERATURE RELATIONS.—Optimum 33° to 34° C. Maximum above 45°. Minimum 5.5°. Thermal death-point about 51°.

COHN'S SOLUTION.—Growth is slight and nonfluorescent. Precipitate stained from a slightly clouded tube showed many long chains. These chains do not always appear.

USCHINSKY'S SOLUTION.—Clouding is moderate to heavy and nonfluorescent. A thin pellicle forms at first which breaks up on shaking. After a week or two the pellicle hangs in long strands down through the liquid from a small surface disk. There were no long chains on stained slides.

FERMI'S SOLUTION.—The clouding is heavier than in either Cohn's or Uschinsky's solution. A thin pellicle forms which is not continuous but like lacework; nonfluorescent. Pellicle and precipitate break up on shaking. There are no chains on stained slides.

BLOOD SERUM.—Growth is moderate, filiform, flat, glistening, smooth. The medium is not liquefied.

MILK.—There is no coagulation. Clearing begins in about a week and is completed in from four to six weeks.

In one test, tubes 2 months old were a maize yellow (Ridgway)[1] turning to brownish at the top. Other tubes 4 months old were a Sudan and Brussels brown (Ridgway).

LITMUS MILK.—In 24 hours a blue rim shows at the top of the milk. In three days the inoculated tubes are all light blue and by the end of a week are colorless.

METHYLENE BLUE WITH MILK.—Tubes of milk containing methylene blue become colorless in 48 hours.

INDOL PRODUCTION.—Tests were made with sulphuric acid and sodium nitrite on cultures grown in Dunham's solution for 1, 2, 4, 5, 9, and 12 days. No indol was produced in this solution, nor in one made up with 0.5 per cent disodium phosphate, 0.1 per cent magnesium sulphate, and 1.0 per cent peptone in 100 cc. of distilled water.

HYDROGEN SULPHID.—Hydrogen sulphid is produced by cultures on gelatin, beef-peptone agar, beef broth, and potato cylinders. The margins of lead acetate paper were slightly darkened over gelatin, broth, and potato cylinders, and turned a dark brown over agar.

AMMONIA.—The production of ammonia is moderate.

NITRATE REDUCTION.—Nitrates are promptly reduced. Tests were made on cultures in nitrate broth with starch water, potassium iodid, and sulphuric acid.

FERMENTATION TUBES.—Cultures I, II, and V were grown in fermentation tubes containing a 1 per cent peptone solution to which was added 1 per cent each of saccharose, lactose, maltose, dextrose, mannit, and glycerin. No gas was formed. Clouding was heavy in the open arm but no pellicle was formed. There was no clouding in the closed arm during the first few days. Tested with litmus paper the inoculated tubes were always alkaline whichever carbon compound was used.

LOSS OF VIRULENCE.—Loss of virulence was slight in cultures carried for two years.

CRYSTALS.—Crystals are formed along the sides of the tubes in Uschinsky's solution, and occasionally in bouillon.

TOLERATION OF ACIDS.—The proso organism grows promptly and well in neutral bouillon containing 0.1 per cent malic, tartaric, and citric acids, (P_H 6.2) but does not grow at all in bouillon containing 0.2 per cent (P_H 5.0–5.2) or 0.3 per cent (P_H 4.5–4.8) of these acids.

LITMUS SUGAR AGAR.—Cultures I and II grown on litmus sugar agar (2 per cent peptone, 1 per cent sugar, 1 per cent agar in distilled water) produced no acid with the following carbon compounds: Saccharose, lactose, maltose, dextrose, galactose, mannit, arabinose, raffinose. Reduction occurred with galactose, arabinose, and dextrose.

OPTIMUM REACTION AND TOLERATION LIMITS IN BOUILLON.—The optimum reaction is +21 (P_H 6.15) to +24 (P_H 6.3). Toleration limits +33 (P_H 5.4) and −22 (P_H 10).

DRYING.—Dried smears on cover glasses usually live for five days, but cloud sterile broth slowly when dropped into it at the end of that time.

FREEZING.—Ninety-nine per cent were killed by freezing in salt and ice for 20 to 30 minutes.

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 col. pl. Washington, D. C. 1912.

SUNLIGHT.—The proso organism is sensitive to direct sunlight—90 per cent were killed by exposure in poured plates on ice for 15 minutes. (Washington, D. C., June 22.)

VITALITY ON CULTURE MEDIA.—The organism is resistant on media. Cultures grown on beef-peptone agar and bouillon in the ice box for 14 months produced good growth on agar and in bouillon in 48 hours.

IDENTIFICATION.—The number of this organism is 211.3332023 according to the descriptive chart of the Society of American Bacteriologists, 1914.

The name *Bacterium panici* n. sp. is suggested for this organism.

TECHNICAL DESCRIPTION

Bacterium panici n. sp.

A motile rod with rounded ends and polar flagella; single or in pairs, occasionally in chains; average measurement 1.66μ×0.69μ; no spores or zoögloea; involution forms occur at high temperatures; capsules are formed; aerobic; on beef-peptone agar colonies are round, white, smooth, shining, raised, margin at first entire and later undulate; gelatin is liquefied slowly; milk is cleared in 5 to 6 weeks without coagulation; litmus milk turns blue in 3 days and reduction takes place in 7 days; ammonia and hydrogen sulphid are produced; indol and gas are not produced; nitrates are reduced; diastasic action on potato cylinders is moderate; growth is slight in Cohn's solution, moderate in Uschinsky's, heaviest in Fermis', and nonflourescent in all three; maximum temperature for growth 45°, minimum 5.5°, optimum 33 to 34° C.; optimum reaction for growth +21 (P_H 6.15)– +24 (P_H 6.3); toleration limits +33 (P_H 5.4) and –22 (P_H 10); gram negative; not acid fast; stains readily with carbol fuchsin and gentian violet and lightly with methylene blue; sensitive to drying; 99 per cent killed by freezing; 90 per cent killed by sunlight; retains vitality on culture media for 14 months; pathogenic on varieties of proso producing narrow, brown, water-soaked streaks on leaf blades, sheaths, and culms.

COMPARISON WITH BACTERIAL DISEASES OF RELATED PLANTS

A. A. Crozier, in a bulletin on millet published at the Michigan State Agricultural College Experiment Station in 1894 (*3*), makes the following statement under diseases: "The sorghum blight (*Bacillus sorghi*) was present in several of our samples of German millet this year, appearing as black streaks in the leaves, but doing no particular damage."

This is the only reference to a bacterial disease of millet found by the writer. It is possible that these are lesions produced by the organism described in this paper, but no work appears to have been done on the disease.

Bacterial diseases of both broom corn and sorghum have been described. In 1887 Burrill described a bacterial disease of broom corn and sorghum (*1*, *2*). The plants were described as being yellow and sickly in appearance with the lower leaves dying first, but the most conspicuous signs of disease were the red blotches of all sizes and shapes on the leaves and sheaths and even on the brush of broom corn. He states that numbers of bacteria were observed microscopically in the diseased tissues, the organism was isolated, and successful inoculations were made from cultures and from macerated diseased tissue. The organism was named *Bacillus sorghi*.

In 1905 Smith and Hedges (*7*) described a bacterial disease of broom corn as occurring on the Arlington Experiment Farm at Washington, D. C., in 1904. They state, "The elongating red blotches were extremely numerous and fused readily, causing the death of many large leaves." Bacterial exudate in the form of red crusts or scabs is described as occurring on the undersurface of the spots. A white organism was

isolated and successful pure culture inoculations made by spraying plants with water suspensions of the organism. Organisms obtained from leaf spots on sorghum produced the characteristic lesions on broom corn. Lesions on broom corn are illustrated in the first volume of Smith's Bacteria in Relation to Plant Diseases (*5, pl. 20*). In Volume II (*6, p. 63–64*) Doctor Smith shows cross sections of leaves illustrating stomatal infection. In a footnote he gives the organism the name *Bacterium andropogoni*, with a brief characterization (*6, p. 63*).

In both diseases on broom corn and sorghum the lesions are described as red blotches of varying size and shape. The reddening is, of course, a host reaction which can also be produced by mechanical or other injury. Proso plants do not react in this way. Reddening does not follow bacterial invasion or other injury, and consequently bacterial lesions are not red but water-soaked and brown. Realizing that it might be possible for one organism to infect plants of closely related genera and produce a different host reaction in each, these diseases of sorghum and broom corn were kept in mind while working with the proso organism. Cultures of *Bacillus sorghi* and *Bacterium andropogoni* were not available for cross inoculations, and unsuccessful attempts were made to infect various kinds of sorghum and broom corn with the proso organism as described above. Twelve inoculations on sorghum, broom corn, and related plants produced no lesions, while proso plants inoculated at the same time and kept under the same conditions developed typical lesions.

A comparison of the cultural and morphological characters of these three organisms brings out important differences. The fact that Burrill's organism was a bacillus precludes further comparison with the polar flagellate organism infecting proso. It produces spores and does not liquefy gelatin, while the proso organism does not produce spores and liquefies gelatin slowly.

Bacterium andropogoni, E. F. Smith, and the proso organism are both polar flagellate, white, slow-growing organisms producing no spores, bluing litmus milk, producing no indol. They differ, however, in important cultural characteristics. *Bacterium andropogoni* is sticky on agar and hard to remove, while the proso organism is butyrous. *Bacterium andropogoni* does not liquefy gelatin and does not reduce nitrates. The proso organism liquefies gelatin slowly and reduces nitrates promptly. These cultural differences, combined with the lack of infection on sorghum and broom corn with the proso organism, lead to the conclusion that the two organisms and diseases are distinct.

DISSEMINATION OF PROSO ORGANISM

During the growing seasons of 1921 and 1922 seed of Early Fortune, was sown at Tuxedo, Md., on ground which had not, as far as known, ever been sown to proso, certainly not for several years. In 1921 the seed was sown late and the season was dry. Only scattered lesions of the bacterial disease appeared on the leaves and peduncles of the plants. Isolations were made, however, which produced the disease on greenhouse plants during the winter. The seed from this plot was planted in 1922 on ground about 200 feet from the 1921 plot. For several weeks after planting there was a great deal of rainy weather, and temperatures and humidity were high. When the plants were about half grown there was 100 per cent infection and about 10 per cent of the plants had been

killed by the disease. In many plants the youngest leaves and growing point of the stem were brown and water-soaked. Many plants showed infection at the crown, being discolored and readily pulling away from the roots.

It seems probable that in both the 1921 and 1922 plots the initial infections came from the seed and in 1922 the rainy weather was responsible for the rapid spread of the disease. Methods of seed disinfection have not been worked out.

SUMMARY

Proso or broom corn millet showing brown, water-soaked streaks on the leaves, sheaths, and culms has been collected at Brookings, S. Dak., and Madison, Wis. These lesions are one to several millimeters wide and from one-fourth to several inches long and show numerous thin white scales of exudate.

A white polar flagellate organism has been isolated from these lesions which readily reproduces the disease when sprayed onto healthy plants. This organism differs culturally and morphologically from organisms attacking related plants and the name *Bacterium panici* is given.

The disease is probably transmitted by seed. No methods of control have been worked out.

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PLATE 1

Early Fortune proso. Head just pushing out. Natural infection with bacterial disease. Madison, Wis., July, 1920. Slightly reduced.

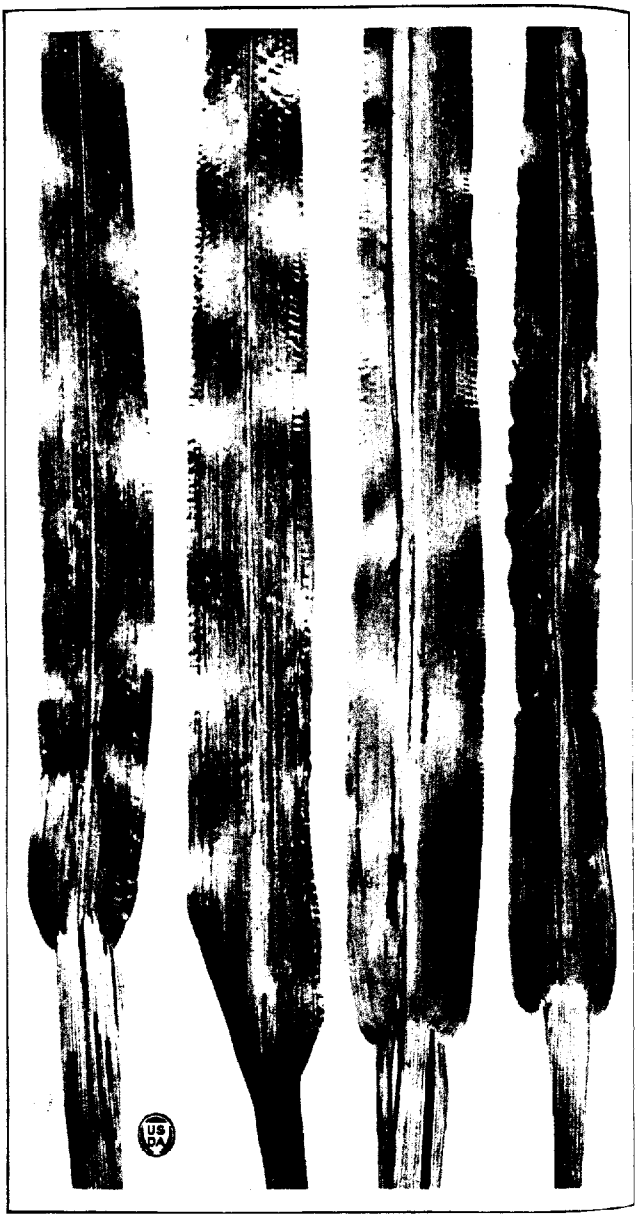


PLATE 2

Early Fortune proso. Natural infection on blades and sheaths. Madison, Wis., ly, 1920. Slightly reduced.

PLATE 3

A.—Natural infection on Early Fortune proso. Note heavy infection on two youngest leaves and the dark central axis which has been killed by the disease. Madison, Wis., July, 1920. Slightly reduced.

B.—Early Fortune proso. Lesions produced by spraying greenhouse plants with culture V, February 25, 1922. Photographed March 4, 1922. ×1½.


A
B

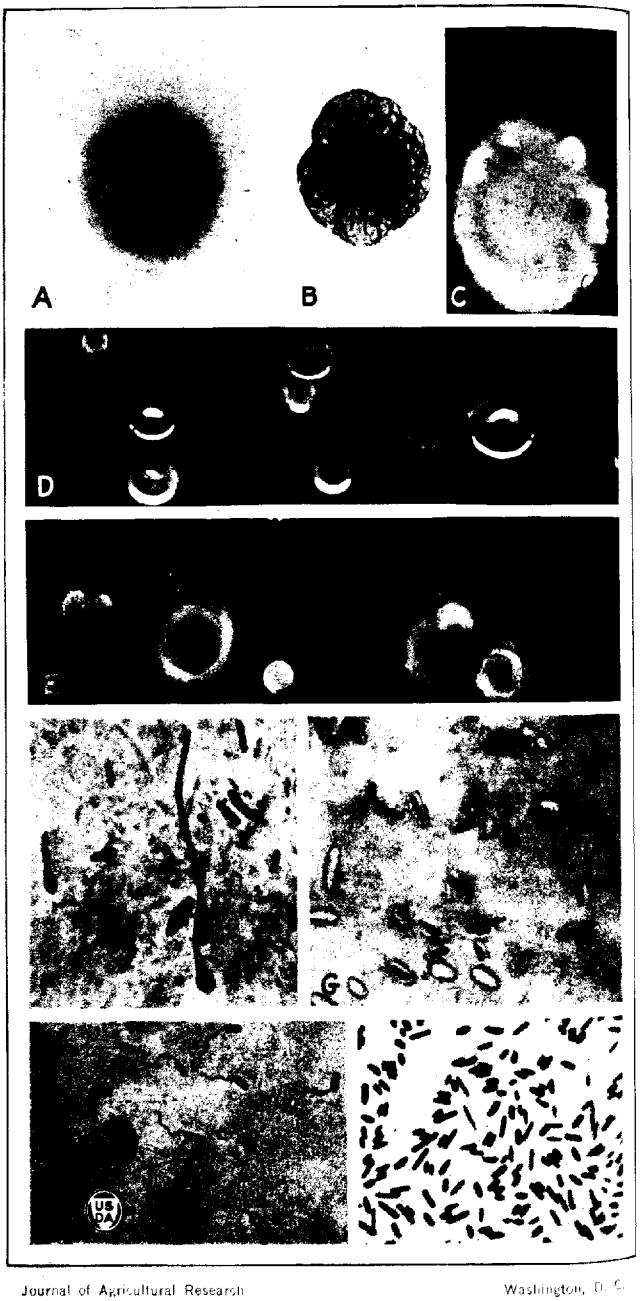

A
B
C
D
E
F
G
USDA

PLATE 4

A.—Culture I, surface colony, 5 days old, on +13 gelatin $\bar{x}491$. × 90. Transmitted ht.

B.—Culture I, embedded colony, 5 days old, on +13 gelatin $\bar{x}491$. × 90. Transitted light.

C.—Culture II, surface colony, 4 days old, on +15 agar showing V-shaped growths margin. × 10. Transmitted light.

D.—Culture I, surface colony, 5 days old, on +13 gelatin showing depressed centers 15. Reflected light.

E.—Culture I, surface colonies, 10 days old, on +13 gelatin showing depressed nters. ×15. Reflected light.

F.—Culture I, involution forms grown for nine days on beef agar at 33° C. × 1,500.

G.—Culture V, beef-agar cultures stained with Ribbert's capsule stain. × 1,900.

H.—Culture II, 3-day-old, on +15 agar. Casares Gil stain. × 1,400.

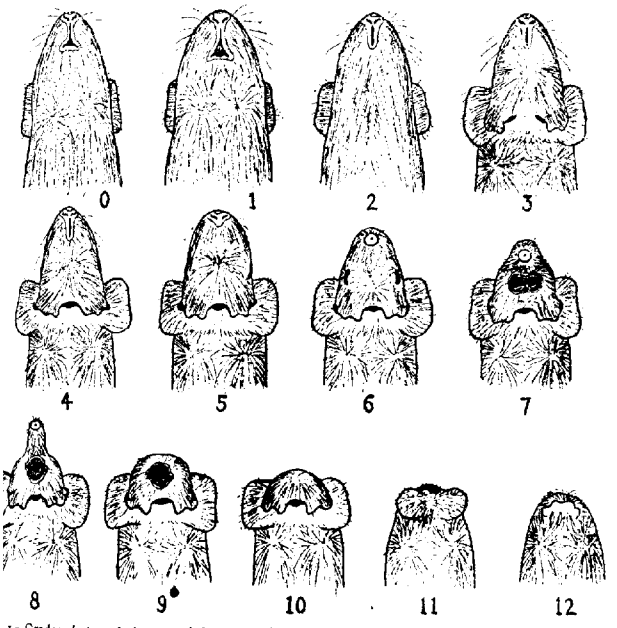
I.—Culture I, one-day culture on potato. Polar staining. Carbol fuchsin stain. 1,700.

ACTORS WHICH DETERMINE OTOCEPHALY IN GUINEA PIGS[1]

SEWALL WRIGHT, *Senior Animal Husbandman in Animal Genetics*, and ORSON . EATON, *Scientific Assistant in Animal Genetics, Animal Husbandry Division, nited States Department of Agriculture*

INTRODUCTION

learly all of the recognized types of monstrosities have appeared in the :k of guinea pigs maintained by the Bureau of Animal Industry for etic experimentation. The most abundant type, unless all those h leg or toe abnormalities are lumped together, has been that which



1.—Grades of otocephaly. Semidiagrammatic ventral views of the head and throat of the 12 grades in comparison with the normal (0).

offroy Saint-Hilaire (7)[2] called "otocephalien" in his classical mono- ph on the subject. Certain of the grades are of the well-known lopean type.

he most characteristic feature of these monsters is the close approach he ears, there being in most cases but a single median opening in the oat. There has been wide variation in the degree of defect, but all cases observed in our stock of guinea pigs fall practically into a single

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eference is made by number (italic) to "Literature cited," p. 180–181.

series of increasing defectiveness. Twelve grades, based on extern appearance, have been used. These are shown somewhat diagrammatically in figure 1. Photographs of certain of the grades are shown in Plate 1, A and B.

GRADES OF OTOCEPHALY

In grade 1 the only obvious defect is more or less reduction of the lower jaw. In grade 2 no mandible can be felt externally. In grade 3 the ears are connected under the throat by bare skin. In grade 4 there is only a single median ear opening on the throat. In grade 5 the mouth and upper incisors are lost. In grade 6 the nostrils fuse. In grade 7 the eyes are in contact below a narrow nasal proboscis or are more or less fused. This fusion is complete in grade 8. The proboscis is lost in grade 9, the eye in grade 10, and the ear opening in grade 11. Two small ears are the only externally visible organs of the head left. In grade 12 the body rounds off in front of the shoulders, with no sign of a head except a single small median external ear. This is the most advanced grade which has been found.

The internal anatomy has been studied to a considerable extent in the various grades, but will not be described in detail in this paper. It may be said, however, that all the changes from grades 1 to 4 are apparently consequent on reduction in Meckel's cartilage. In grade 4 the mandible is a short, flat crescent of bone firmly united to the reduced tympanics and hence to the upper part of the skull. The zygomatic arches are in contact or more or less fused posteriorly. The ear ossicles are fused and concealed by the reduced mandible. There is a mouth cavity, bounded ventrally by a mass of muscle in place of the lower jaw. The pharynx is necessarily very narrow in passing between the upper part of the skull above and the fused zygomas, and the fused ear ossicles and reduced mandible below. It expands posteriorly into the single middle ear. Posterior to this a swelling in the floor represents the tongue.

A new series of changes begins with grade 5, in which the zygomas fuse anteriorly as well as posteriorly and the tooth-bearing portion of the maxillaries is lost. The cause is probably arrested development in the fronto-nasal process. Beginning with grade 6, changes in the brain become well marked. The cerebral hemispheres fuse in grade 6, though retaining about the normal shape and size. The fusion spreads back to the optic chiasma in grade 8, and there is continually increasing reduction in size of the fore-brain sac relative to the cerebellum in grades 7, 8, 9, and 10. The median optic nerve of grades 8 and 9 is lost in grade 10. In the specimen of grade 11 which was examined nothing was left of the brain but the medulla. The skull was reduced to a fairly normally shaped but undersized occipital ring posteriorly and normally sized but distorted periotic capsules anteriorly, with four minute flat bones (interparietal, parietals, fused frontals) between the latter. The persistence of the parts of the inner ear—cochlea and semicircular canals—is noteworthy. The fenestrae, however, were found to be absent in all specimens in which the ear ossicles were fused—that is, in some specimens of grade 3 and all of higher grades. The body ordinarily is plump and apparently normal, even in the most advanced grades. No internal abnormalities have been found in the body.

HISTORY OF THE GUINEA PIGS

The distribution of these otocephali by grade among the inbred families l other related experiments is shown in Table I. The inbred families ve been maintained since 1906 wholly by matings of brother with ter except for Family 4, in which parent-offspring matings were the e (*15, 16*). The foundation pairs of most of them (No. 1 to 24) were en from a stock which had been maintained since 1894 by the Bureau Animal Industry without the introduction of fresh blood. The females Families 31 to 39 came from this stock, while the males were bought m a dealer. At present only five families (2, 13, 32, 35, and 39) are ng maintained.

Stock B represents the original stock, maintained with careful avoid- e of matings closer than third cousins. Numerous crosses have been de among the families.

n addition to the 82 octocephali shown in Table I and subsequent les, there are records of two of unknown grade in an early experiment which full data are not available.

DISTRIBUTION AMONG INBRED FAMILIES

The considerable number of otocephali which have appeared in the red families at first seems to support the old belief that inbreeding lf leads to the appearance of monsters. Further consideration, how- r, throws doubt on this conclusion. We find that 14 inbred families, h a total of 12,037 young, produced no otocephali at all, while 50 of 82 appeared in one family, No. 13. This family produced 1.54 per t, while only 0.11 per cent appeared in all other inbred families, taken ectively.

LE I.—*The number of otocephali of each grade among 24 inbred families, crosses involving these families, and a random-bred control stock, through June, 1922*

Family No.	Grades of otocephali.											Total otocephali.	Total young.	Per cent otocephali.
	1	2	3	4	5	6	7	8	9	10	11			
.....	...	...	...	1	...	...	...	...	...	...	...	1	558	0.18
.....	...	...	...	2	...	1	...	...	...	...	...	3	1,182	.25
.....	...	...	...	...	...	...	...	1	...	...	...	1	1,160	.09
.....	2	5	6	26	2	1	1	1	2	3	1	50	3,253	1.54
.....	1	...	...	1	...	...	...	...	...	...	...	2	1,308	.15
.....	...	1	...	...	...	...	...	...	...	1	...	2	880	.23
.....	...	...	1	3	...	1	...	...	...	...	...	5	537	.93
.....	...	3	2	2	...	...	...	1	1	...	...	9	2,718	.33
.....	...	...	...	...	...	1	1	2	...	...	...	4	3,027	.13
ther families.....	...	...	...	...	1	...	...	...	...	...	...	1	1,461	.07
l inbreds.....	...	...	...	...	...	...	...	...	...	...	...	0	12,037	.00
eds, excluding mily 13.....	3	9	9	35	3	4	2	5	3	4	1	78	28,121	.28
ses.....	1	4	3	9	1	3	1	4	1	1	0	28	24,868	.11
k B.....	...	...	...	2	1	...	...	...	...	...	...	3	6,659	.05
	...	...	...	...	1	...	...	...	...	...	...	1	4,495	.02
Total.....	3	9	9	37	5	4	2	5	3	4	1	82	39,275	.21

Inbreeding resulted in a decline in vigor on the average in all respects, including the weight at birth and later ages, size and frequency of litters, and the mortality at birth and later. The families differed greatly in the degree of the decline. It might be thought that the families which declined the most in vigor would produce the most monsters. It happens, however, that Family 13, with by far the highest percentage of otocephali, was also the most vigorous on the whole of the 23 families up to 1915.[4] It has always been among the best three in weight at all ages and in size of litter, and is the only family which was better than the average in all respects studied. Among the five families now on hand it still produces the heaviest pigs and the largest litters. The two weakest families in nearly all respects were No. 1 and 15, which produced no otocephali or monsters of any sort except for one clubfoot (ectromelus) in Family 15. It is clear that the production of otocephali is not merely a manifestation of lack of vigor.

The conclusion which is forced upon us is that there is an important hereditary basis to otocephaly. The part which inbreeding plays here, as in other cases, is merely to bring clearly to light the hereditary differences between different strains. Indeed, the fact that one high-grade otocephalus appeared in the control stock B indicates that the tendency was probably present before the inbreeding commenced.

DISTRIBUTION BY GENERATIONS

The distribution of the otocephali within Family 13 and within Family 32, which stood second in their production, is of interest. In the former the first otocephalus appeared in 1908, in the second generation of inbreeding, but there were no others until one came in the eighth generation in 1912. Twenty-five of the 50 in this family appeared in the seventeenth and later generations and no fewer than 17 in the two most advanced generations, the twentieth and twenty-first. This again suggests the traditional cumulative effect of inbreeding. The history of Family 32, however, happens to be nearly the reverse. The nine otocephali were produced in the third to the eighth generations among 1,094 young. Not one has appeared since the eighth generation (1,516 young) in contrast with 48 in Family 13 in these generations, although the two families have run roughly parallel in number of young and generations of inbreeding. Family 32 has now (1922) reached the twenty-first generation. The other families show no tendency toward increasing appearance of otocephali. Only 1 out of 19 has appeared since the thirteenth generation, although one of these families has reached the twenty-fourth generation.

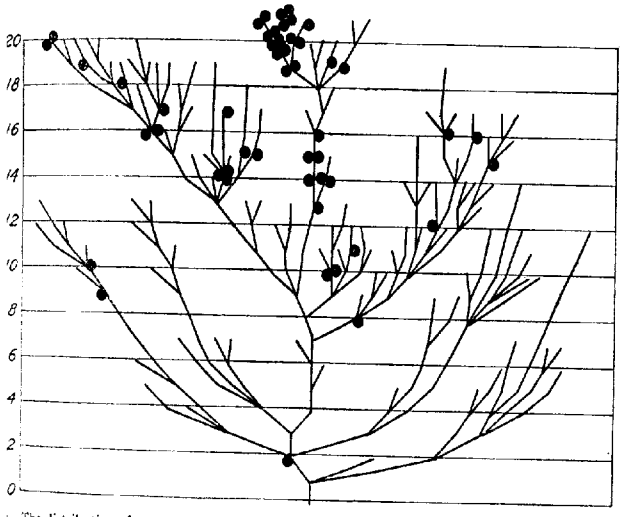
Under the system of brother-sister mating, each family tends to split up into subfamilies. The subfamily which happens to advance most rapidly in inbreeding tends to displace the others. The apparently contradictory results in Family 13 and the others, relative to distribution by generations, are readily reconciled by the hypothesis that there is segregation of the hereditary factors for otocephaly, and that it is largely a chance whether the subfamily which receives such factors to the greatest extent is the one which pushes ahead most rapidly.

[4] The data for Family 4 have not been analyzed.

DISTRIBUTION WITHIN FAMILY 13

A detailed study of the history of Family 13 brings out additional nts of interest. The lines of descent are shown in figure 2. The cephali are represented by circles.

The features which stand out in the chart are the clusters of otocephali certain lines and their absence in other important subfamilies. The ginal female of Family 13 was mated twice. No otocephali appeared ong the 404 descendants of her first mate (a line not shown in figure All the otocephali descend from a mating in the second generation he other line (13–2–5). Another second-generation mating (13–2–7) 199 descendants, but none were otocephalic. Four subfamilies may recognized as springing from 13–2–5 in the third or fourth generation.



—The distribution of otocephali among the matings of Family 13. Since all matings have been ween brother and sister, each pair traces back through a single line of matings to the foundation shown at the bottom of the family tree as the 0 generation. The black circles represent the cephali. Note the difference in frequency in different branches of the family.

of these (13–4–9, 13–3–13) with 196 and 301 young, respectively, duced no otocephali. One with 150 young (13–3–11) produced two an interval of seven generations from 13–2–5. The other sub- ily (from 13–4–18) was a very small one in the early generations. It duced its first otocephalus in the eighth generation. It began to and with the seventh generation until it displaced all other lines. its branches produced otocephali relatively freely in comparison other inbred families. The general tendency seems to have been production of about 1 per cent. At several points, however, branches e in which the tendency seemed to increase to a noteworthy extent. first cluster of this kind traces to 13–13–1. This mating and its endants, even excluding the most prolific branch, of which we will e more to say, produced 9 among 212 young, or more than 4 per cent. veritable epidemic of otocephaly began with mating 13–19–1 in the h generation from 13–13–1. The six matings of this group have

produced 17 of the monsters among 79 young,[4] or 21.5 per cent. A third fairly noteworthy cluster takes its origin in mating 13–16–3 from which nearly 3 per cent have been otocephali. This line has a common ancestor with 13–13–1 only in the ninth generation. (Table II.)

TABLE II.—*Number and percentage of otocephali among the descendants of particular matings in Family 13. The mating is designated by the generation followed by an arbitrary number*

Matings of Family 13.	Descendants.			Excluding mating 19–1 and descendants.			Excluding mating 13–1 and descendants.		
	Otocephali.	Total young.	Per cent otocephali.	Otocephali.	Total young.	Per cent otocephali.	Otocephali.	Total young.	Per cent otocephali.
0–1	0	404	0	.....	.....	.....	.....	.....	.....
0–2	50	2,849	1.8	33	2,770	1.2	24	2,558	0.9
2–6, 7	0	201	0	.....	.....	.....	.....	.....	.....
2–5	50	2,636	1.9	33	2,557	1.3	24	2,345	1.0
3–13	0	301	0	.....	.....	.....	.....	.....	.....
3–11	2	150	1.3	.....	.....	.....	.....	.....	.....
3–12	47	2,168	2.2	30	2,089	1.4	21	1,877	1.1
4–9	0	196	0	.....	.....	.....	.....	.....	.....
4–18	47	1,960	2.4	30	1,881	1.6	21	1,669	1.3
8–11	5	625	0.8	.....	.....	.....	.....	.....	.....
8–3	42	1,291	3.3	25	1,212	2.1	16	1,000	1.6
13–7	13	637	2.0	.....	.....	.....	.....	.....	.....
16–3	7	263	2.7	.....	.....	.....	.....	.....	.....
13–1	26	291	8.9	9	212	4.2	.....	.....	.....
19–1	17	79	21.5	.....	.....	.....	.....	.....	.....

Summing up, we may say that of six early sublines of Family 13, four showed no tendency to produce otocephali, while the other two probably soon acquired, if they did not start with, a tendency to produce about 1 per cent. One of these became the main line of the family and gave rise to several important branches, all of which showed the tendency to an unusual extent. A sudden jump to about 4 per cent seems to have occurred at some point in one subline, with a second jump in the same line, six generations later, to more than 20 per cent.

The distribution within Family 13 adds to the evidence for heredity as an important basic factor. The mechanism of this heredity, however, is far from clear.

A single recessive factor is as much out of the question as a dominant factor. The ratio of normals to otocephali is 493 to 50 (9.9 to 1) in Family 13, and 346 to 32 (10.8 to 1) in other families within those matings which produced at least one of the monsters. It can not be doubted that a great many other matings have an equally strong tendency. In fact, 43 of the 58 matings included above produced only one otocephalus each, 11 produced only two each, leaving only 4 which have produced three or more. If each mating had produced several hundred young instead of about 16 on the average, there can be no doubt that the ratio would be nearer to 100 to 1 than 10 to 1, except in the small cluster of matings descended from 13–19–1.

[4] Ten more otocephali and 36 normal young have been produced by this line since this tabulation, i. e. from July, 1922, through September, 1923, making a total of 27 otocephali out of 125 young, or 21.6 per cent.

It may be thought that the cooperation of a large number of factors s necessary for the appearance of the defect and that only in the luster mentioned has homozygosis been reached in a sufficient number f these factors to give one or two factor ratios. This hypothesis, how-ver, is untenable in view of the system of mating. Brother-sister nating leads automatically to rapid increase in homozygosis. After 10 enerations less than 6 per cent heterozygosis should be left. If oto-ephaly is due to the cooperation of many factors, inbreeding should ead rapidly to the disappearance of otocephali in the great majority of ines, through homozygosis in some one or more of the normal allelo-norphs, while the few lines in which all the factors for otocephaly persist hould be soon producing 3 :1 ratios because of homozygosis in all but ne of the factors. The history of Family 13 is wholly at variance with hese conclusions.

There is one mechanism by which rapid increase in homozygosis would e prevented. If a necessary factor for otocephaly were linked with one thal factor and balanced against another, it could be carried on indefi-itely in a heterozygous condition, otocephaly only appearing on the ccurrence of a crossover. By complicating the situation with other thals the observed results could readily be explained, including the udden increases in the percentage at various points in the pedigree.

Unfortunately other considerations make it very doubtful whether the ssumption of balanced lethals is tenable. With balanced lethals the ize of litter should be greatly reduced (halved except for the higher ercentage of embryos absorbed in large litters). Family 13, as pre-iously stated, has consistently been among the best inbred families as gards size of litter. During the period from 1916 to 1921, in which the ajority of its otocephali were produced, it was the best of the inbred milies. Its average in these years has been 2.53, as compared to 2.33 in e total inbred stock and 2.65 in the control stock. Moreover, with ifferent systems of balanced lethals among the inbred families, one ould expect an increase in the size of litter when a female of one family mated with a male of another or with a crossbred male. No such crease has taken place in extensive and carefully controlled experiments. here is indeed an important increase (about 12 per cent) when the cross-ed daughters of such matings are themselves mated, whether with a other, an unrelated inbred, or a crossbred. But this indicates that size litter is determined by the breeding of the dam, and not of the young emselves, as should be the case with balanced lethals.

If the otocephali are not Mendelian segregates the possibility must be nsidered that they are mutations or due to chromosome aberrations of me sort. The sporadic occurrence outside of Family 13 is reasonably harmony with this view. The number and distribution in Family 13, wever, can not be explained satisfactorily in this way. It seems clear at a genetic factor or factors for otocephaly must be transmitted by rmals in Family 13.

Thus neither Mendelian segregation, even with balanced lethals, nor utation, is a satisfactory explanation by itself of the observed distribu-n. Under both these explanations it is assumed that the otocephali a group differ genetically from all normals. But even if the difficulties th these purely genetic explanations were less, the continuous grada-n from a condition which can not certainly be distinguished from rmal (grade 1) to the almost completely headless condition of grades and 12, should lead us to suspect that some guinea pigs, at least, with

the genetic constitution of an otocephalus, would yet be normal and would live and breed, while the genetic considerations lead us to believe that all animals in a given advanced subline of an inbred family are of the same or very nearly the same genetic constitution. We can assume in harmony with the experimental results of Dareste (*2*), Stockard (*8*, *9*), Lewis (*3*), Werber (*11*), and others that the actual occurrence of an otocephalus or cyclopean within such a line is due to particular environmental conditions. The part which genetic factors play is to determine differences in the susceptibility to such conditions. There is segregation of different degrees of susceptibility in the early generations of such a family as No. 13, followed by the relative fixation of a particular level in each subline as some combination of favorable factors becomes homozygous. Thus lines starting from matings 13–3–11 and 13–4–18, in which about 1 per cent develop into otocephali under the prevailing conditions, segregated from the lines starting from 13–2–7, 13–3–13, and 13–4–9, in which none developed.

The sudden jumps in the percentage of otocephali starting from 13–13–1, 13–19–1, and perhaps 13–16–3 can probably best be interpreted as mutations. The experiments of Dareste, Stockard, and others have shown that otocephaly or the closely related cyclopean condition is a defect which can be brought about by a great diversity of agents, temperature, low oxygen pressure, magnesium salts, butyric acid, and even mechanical disturbance. Apparently anything which depresses metabolism sufficiently at a certain critical moment in development acts most drastically on the sensitive anterior end of the central nervous system resulting in this type of monster. It is to be expected that the genetic factors which determine high or low resistance to such conditions would also be highly general in nature. Any factor which influences the level of metabolism at the critical moment should have the same effect. The mutations which influence the tendency toward otocephaly should be relatively numerous. Most of them should increase the tendency on the principle that a chance mutation is more likely to disturb normal adjustments than to improve them. Thus the pedigree of Family 13, with its 1 per cent tendency starting from 13–2–5, jumping to a 4 per cent tendency in one subline 11 generations later, and this subline jumping to more than 20 per cent in a branch six generations later, is just what should be expected.

The distribution in other families, such as No. 19 and 32, in which the otocephali occurred only in early generations, is explainable as the result of early segregation and chance displacement of the susceptible lines by resistant ones. Family 13 might have had this history if it had been the descendants of 13–3–13, which had multiplied most rapidly, instead of those of 13–4–18.

THE RESULTS OF CROSSES

If there are genetic differences only between different inbred lines, not within them, it is not an easy matter to learn much of the details of their inheritance. There is one point of considerable importance, however, on which there is some evidence. It is conceivable that otocephaly may be a maternal character so far as its hereditary basis is concerned. That which is inherited may be a tendency toward faulty implantation of the ovum (the factor Mall considered most important) or a tendency toward the production of toxic metabolic products with

ıbsequent injury to the offspring (as suggested by Werber (*11*) in ınnection with experiments on butyric acid and acetone).

If the genetic element is of any such kind, females of Family 13 should :oduce as high a percentage of otocephali in outcrosses as in matings ith brothers. A large number of outcrosses of this kind have been ade, all of them since 1916, since which time Family 13 has been ɔmposed exclusively of high producing lines and has produced an verage of 2.7 per cent otocephali. There have been 711 young from osses in which the dam was of Family 13 and the sire either an inbred a cross between two other inbred families. One otocephalus appeared, frequency of one-seventh of 1 per cent, where some 19 were to be ɛpected on the basis of the production of such females mated with rothers during this period. The single otocephalus was sired by a ıale from a cross between Families 32 and 39, the former of which :oduced 9 otocephali itself.

Among 373 young whose sire was of Family 13 but whose dam was of nother family or unrelated cross, there were no otocephali. There was lso none among about 3,000 crossbred young, neither of whose parents ad blood of Family 13. Two, however, were produced by crosses in hich Family 13 was involved on both sides. One of these was a three-uarter-blood, dam of Family 13 and sire from a cross between Families 3 and 34. There have been 147 three-quarter-blood young whose ams were of Families 13 and 189, none otocephalic, from the reciprocal ɣpe of mating. The other crossbred otocephalus was from a selection ɛperiment (CL). Both the sire and dam were one-quarter blood of amily 13. Among 234 F_2 young from crosses between Family 13 and her families, there were no monsters of this kind.

This study of the crosses again indicates transmission of the otoce-halic tendency from Family 13. It also indicates that it is not a aternal character.

SEX

Table III shows data on the sex ratio among otocephali. Both in Family 3 and in the other families there have been more than twice as many ımales as males. In all there have been 55 females and 26 males with undetermined, a sex ratio of 47.3 as compared with approximate ɉuality among all the young from the same matings (sex ratio 97.0). here is here a departure of 4.8 times the probable error. Such a de-arture would occur only about once in 800 times by chance. It is ıus fairly certain that female sex predisposes toward development of ıis defect. A possible explanation is that the level of metabolism is wer in female than in male embryos at the critical moment in develop-ıent, rendering them more easily depressed by unfavorable conditions. ıch an interpretation is in harmony with the views of Whitman and iddle (*12*) on the early differentiation of the sexes.

TABLE III.—*The number of males, females, and young of undetermined sex, and the sex ratio among the otocephali in the litters and from the matings containing the latter (otocephali included in the latter classes)*

	Family 13.				Others.				Total.			
	♂	♀	?[a]	Sex ratio.	♂	♀	?[a]	Sex ratio.	♂	♀	?[a]	Sex ratio.
				Per cent.				*Per cent.*				*Per cent.*
Otocephali.....	16	33	1	48.5	10	22	0	45.5	26	55	1	47.3
Litters with otocephali.....	55	66	4	83.3	44	48	0	91.7	99	114	4	86.8
Matings with otocephali.....	253	279	11	90.7	194	182	2	106.6	447	461	13	97.0

[a] Undetermined.

ENVIRONMENTAL CONDITIONS

The data have been studied carefully for indications of the effects of environmental factors. The size of litter, birth weight, and mortality at birth and from birth to weaning are characters which are greatly affected by external conditions. The month of birth is important, since conditions have usually been distinctly poorer in winter than during the rest of the year. The possibility that birth rank (first, second, third litter, etc.) may play a part has also been investigated.

In these studies a control is necessary. The averages for the characters listed above have varied greatly from year to year in all stocks depending largely on how the guinea-pig colony has passed through the winter. There has also been a decline, due to inbreeding. Thus the records of stocks producing young at approximately the same time should be used as a control. The best plan has appeared to be to compare the otocephali with their litter mates and both of these classes with the young produced by other litters from the same matings.

Family 13 has been dealt with separately from the other stocks. It will be noticed that its records are usually somewhat lower. This may appear to contradict the statement previously made that Family 13 was the most vigorous of the inbred families, notably in size of litter and weight. The explanation is that most of the otocephali of this family came in the later years, when there had been a decline due to inbreeding and, in certain of which, conditions were at the poorest (as judged by the control stock) while most of the other otocephali came in early years when there was less inbreeding and conditions were better. In any given year, Family 13 stands out as the best of the inbred families in these respects.

BIRTH RANK

Table IV summarizes the data on birth rank, showing the birth rank of otocephali in relation to that of all young from matings which produced them, with the omission, however, of two matings which produced only one litter each (containing five young, of which three were otocephali). All matings included produced at least three litters. A fair comparison can accordingly be made of the percentage of otocephali in first, second, and third litters. A slightly smaller percentage is to be expected in the fourth and later litters for obvious statistical reasons.

TABLE IV.—*The birth rank of otocephali in relation to that of all young from matings which produced otocephali, excluding two matings which produced only one litter each*

	Rank of litters.												Total.	Last recorded.
	1	2	3	4	5	6	7	8	9	10	11	12		
Otocephali.....	17	11	17	10	6	7	3	1	4	1	1	1	79	10
Total.....	145	155	161	136	103	80	48	36	23	15	10	4	916	165
Per cent.....	11.7	7.1	10.6	7.4	5.8	8.7	6.3	2.8	17.4	6.7	10.0	25.0	8.6	6.1

The impression which one obtains from the table is that birth rank is of very little importance. An otocephalus is about as likely to be born in one litter as another. It is true that first litters show some excess over second litters, but the excess over third litters is insignificant. The percentage in last litters from these matings (third to twelfth litter) was a trifle less than in the whole population (6.1 per cent as compared with 8.6 per cent).

First litters are born predominantly in winter when conditions are unfavorable, the majority of matings being made in summer, half a year earlier. Any slight tendency toward excess of otocephali in first litters may be due to this cause. We conclude that there is no demonstrable difference due to birth rank.

SEASONAL FLUCTUATIONS

The number of otocephali born in each month in comparison with all young from the same matings is shown in Table V. It will be noticed that there is no very conspicuous difference among different months, but that a somewhat higher percentage of otocephali are born in the months from January to April, inclusive, than during the rest of the year. During these four months Family 13 produced 42 per cent of its otocephali while the same matings were producing only 27 per cent of their total young. Similarly, the other stocks produced 44 per cent of their otocephali while only 30 per cent of their young were being born. The application of the χ^2 test to the number born in each month, assuming 8.9 per cent as the expected figure in each case, gives the probability of 0.363 that the distribution is random. If, however, the year is broken into four-month periods for application of this test, the probability becomes only 0.013. Thus there is a distinct indication that more otocephali are born following the unfavorable winter conditions than in the rest of the year, but it is only an indication.

TABLE V.—*The month of birth of otocephali in relation to that of all young from matings which produced otocephali*

	Month of birth.												Total.
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Otocephali.....	8	11	7	9	7	5	6	5	8	6	3	7	82
Total.....	55	83	71	50	84	88	86	89	80	67	70	98	921
Per cent.....	14.5	13.3	9.9	18.0	8.3	5.7	7.0	5.6	10.0	9.0	4.3	7.1	8.9

It is interesting to compare the monthly percentages of otocephali with the monthly averages in size of litter and in percentage of the normal young raised to weaning, among the young from the same matings. Size of litter and mortality before weaning are characters which are known to be affected to a marked extent by seasonal conditions. The monthly averages for size of litter, percentage of young not otocephalic, and percentage of normals raised to weaning are shown graphically in figure 3. In order to smooth out the irregularities due to small numbers, the figures for each month were combined with those for the preceding and the following month and averaged. These smoothed averages are shown by the heavy lines. The three curves show considerable similarity. In order to bring this out more clearly, however, it is necessary to compare the percentage not otocephalic with the size of litter about half a month later and the percentage raised of the preceding month. The curves are shoved over in this way in figure 3. Such relations are not unexpected. Size of litter is presumably largely determined by conditions two to three months before the litter is born, the gestation period being about two months and a week. Development as an otocephalus must be determined very early in development, probably before the appearance of the medullary plate, from the experiments of Stockard and others. Assuming that the condition of the dam has an influence, poor conditions should result in otocephali a little earlier than in small litters, but an effect on the mortality of the young should appear still earlier. The conditions during the month preceding birth are probably most important in this connection, their cumulative effect determining the mortality at birth and between birth and weaning. Most of those which die before weaning are unthrifty from the first.

The correspondence between the monthly fluctuations shown in the figure, after allowing for a reasonable lag in each case, adds considerably to the evidence that seasonal conditions play a part, if not a very great one, in determining the occurrence of otocephali.

SIZE OF LITTER

As just pointed out, size of litter is to some extent an indicator of favorable or unfavorable conditions at the time of conception and shortly before. We have just seen that smallness of litter and percentage of otocephali show parallel seasonal fluctuations with a lag of only about half a month. It is interesting to make a direct comparison between the size of the litters in which otocephali were born with the size of those in which normals were born. Data are given in Table VI for otocephalus-producing matings. The number of otocephali and the number of normals in each size of litter is shown, together with an average. This average gives the size of litter relative to individuals, and so is considerably larger than when the litter is taken as the unit, as in figure 3. Both in Family 13 and in the other stocks otocephali were born in smaller litters than normals, and in the former case the difference is 4.7 times its probable error, although only 1.5 times in the latter. In both combined there is a difference of 4.4 times the probable error. There can be little question that conditions which determine small litters are relatively likely to cause otocephalic development. The importance of such conditions, however, is not great.

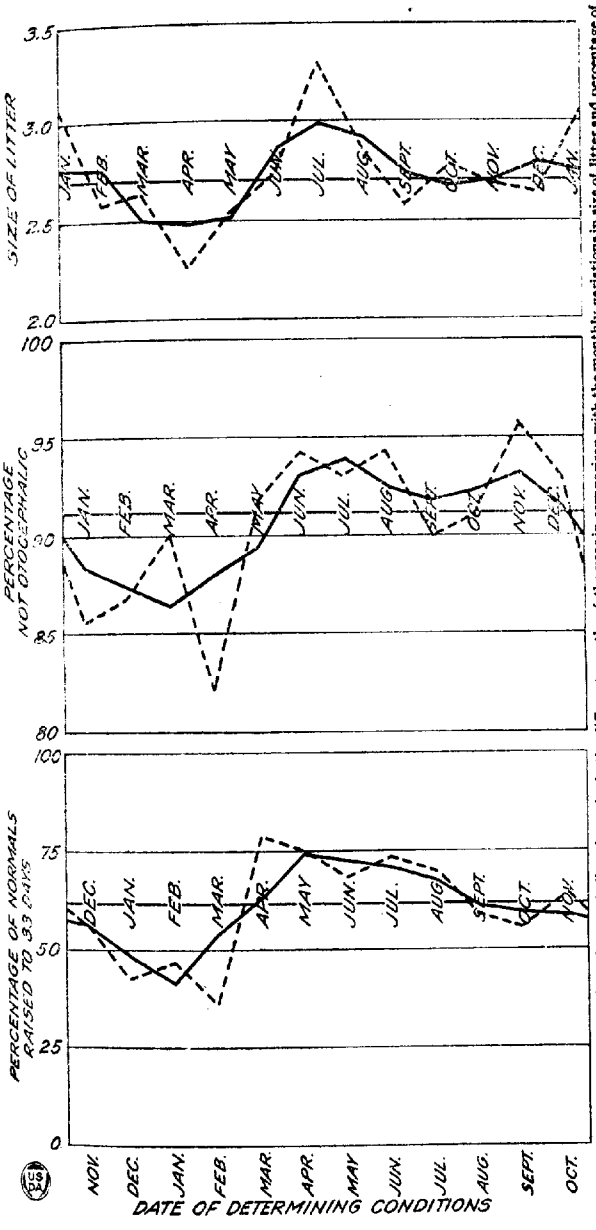


FIG. 3.—The frequency of the birth of otocephalic guinea pigs in the different months of the year in comparison with the monthly variations in size of litter and percentage of normals raised to weaning. The dotted lines show the actual monthly averages, while the solid lines show the smoother three-month averages. All the averages are taken from those matings which produced at least one otocephalus. There is a shift of the months in the three graphs to allow for the different lengths of time between the determining condition and its observed consequences in the three cases. It is assumed that the conditions in a given month have their maximum effect on the mortality of the young born about a month later but determine the occurrence of otocephali two months later and affect size of litter two months and a half later. The gestation period averages 68 days.

TABLE VI.—*The sizes of the litters in which otocephali were born, as compared with those of their normal brothers and sisters* [a]

Size of litter—	Family 13.		Others.		Total.	
	Otocephali.	Normal.	Otocephali.	Normal.	Otocephali.	Normal.
1	3	26	1	15	4	41
2	17	103	9	79	26	182
3	19	173	13	134	32	307
4	11	141	8	72	19	213
5	.....	50	.....	35	.....	85
6	.....	.....	1	11	1	11
Total	50	493	32	346	82	839
Average	2.76±.08	3.17±.03	3.00±.12	3.19±.04	2.85±.07	3.18±.03
Difference	0.41±0.088		0.19±.125		0.33±.075	

[a] Note that the average size of litter relative to individuals is larger than the average with the litter taken as the unit. The average size of litter (litter as unit) was 2.70 and 2.74 in the matings which produced otocephali in Family 13 and other stocks, respectively, as compared with 3.14 and 3.15 where the individual was the unit.

MORTALITY OF LITTER MATES

If otocephali are caused by unfavorable conditions, we should expect a higher mortality among their normal litter mates than among normals from litters which did not include otocephali but which were from the same matings. The parallelism in the seasonal fluctuation, in these characters, allowing a lag of a month, however, has already been shown. The data for the direct relationship between them are presented in Tables VII and VIII. A comparison between Family 13 and other stocks is given in Table VII. In Table VIII all stocks are combined. We find that a distinctly smaller percentage of litter mates were born alive (73.3 ± 2.6 per cent, as compared with 80.7 ± 1.0 per cent for nonlitter mates). Similarly, a smaller percentage of litter mates are raised of those born alive (73.7 ± 3.0 per cent, as compared with 77.3 ± 1.2 per cent for nonlitter mates). Among the litter mates 54.1 ± 2.9 per cent of all young born dead or alive were raised to weaning, as compared with 62.4 ± 1.2 per cent among nonlitter mates.

One objection which may be raised to these comparisons is that they are based on young born in litters of different sizes. There can be, for example, no litter mates of otocephali born in litters of one. If, however, the percentages are compared for corresponding sizes of litter, the same results are found. If the averages for each size of litter among the nonlitter mates are weighted by the number of litter mates born in each size, we get grand averages only slightly different; 81.6 per cent born alive, 77.2 per cent raised of those born alive, and 62.3 per cent raised of all young. The difference between litter mates and nonlitter mates in total percentage raised is 8.3 ± 3.1, or 2.6 times the probable error. There can be little doubt that conditions which cause a high mortality among normal young have some influence in determining otocephaly. Here again, however, the relation is not very great.

TABLE VII.—*A comparison between the mortality figures in Family 13 and the other families which produced otocephali*

	Normal litter mates of otocephali.						Normal sibs, not litter mates of otocephali.					
	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.
	Number.	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*
Family 13.....	34	21	20	73.3	61.8	45.3	244	78	96	77.0	75.8	58.4
Others.....	39	5	16	73.3	88.6	65.0	195	51	40	86.0	79.3	68.2

TABLE VIII.—*The number raised, dying between birth and 33 days, and born dead, and the per cent born alive, raised of those born alive, and raised to 33 days, among the litter mates of the otocephali* [a]

Size of litter.	Normal litter mates of otocephali.						Normal sibs, not litter mates of otocephali.					
	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.	Raised.	Died.	Born dead.	Born alive.	Raised of born alive.	Raised.
	Number.	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*	*Number.*	*Number.*	*Number.*	*Per cent.*	*Per cent.*	*Per cent.*
1.....	.....	.....	.....	.....	.....	.....	23	7	11	73.2	76.7	56.1
2.....	18	3	1	95.6	85.7	81.8	113	24	23	85.6	82.5	70.6
3.....	32	14	9	83.6	69.6	58.2	178	41	33	86.9	81.3	70.6
4.....	19	9	25	52.8	67.9	35.8	82	37	41	74.4	68.9	51.3
5.....	.....	.....	.....	.....	.....	.....	40	18	27	68.2	69.0	47.1
6.....	4	0	1	80.0	100.0	80.0	3	2	1	83.3	60.0	50.0
Total.....	73	26	36	73.3	73.7	54.1	439	129	136	80.7	77.3	62.4
Probable error of total.....	.....	.....	.....	2.6	3.0	2.9	.....	.....	.....	1.0	1.2	1.2

[a] If the percentages for the normals which were not litter mates are weighted by the number in each size of litter among the litter mates, we get 81.6 per cent born alive, 77.2 per cent raised of those born alive, and 62.3 per cent raised, figures which do not differ appreciably from the actual percentages.

BIRTH WEIGHT

Unfavorable conditions naturally have a great influence on birth weight. The average birth weight of otocephali, their litter mates, and their brothers and sisters which were not in litters containing otocephali are given in Table IX. Because of the very important effect of size of litter on birth weight, a correction must be made in order to make valid comparisons. The correlation between birth weight and size of litter (individual the unit) came out -0.41, -0.60, and -0.58 in these three classes of young. The regressions of weight on size of litter deduced from these figures are -8.2, -11.1 and -10.4 gm. per unit difference in size of litter. All average birth weights were adjusted to an average litter of three by use of the regression of -10.3.

TABLE IX.—*The average weight at birth of otocephali, their litter mates and their brothers and sisters of other litters, in Family 13, other stocks, and the total* [a]

	Family 13.				Other stocks.				Total.				
	Number.	Average size of litter.	Average weight.		Number.	Average size of litter.	Average weight.		Number.	Average size of litter.	Average weight.		Standard deviation.
			Actual.	Adjusted.			Actual.	Adjusted.			Actual.	Adjusted.	
			Gm.	*Gm.*			*Gm.*	*Gm.*			*Gm.*	*Gm.*	*Gm.*
Otocephali	50	2.76	66.7	64.2	31	3.00	71.3	71.3	81	2.85	68.5	67.0±1.4	18.7
Litter mates	75	3.27	71.3	74.1	60	3.43	73.0	77.4	135	3.34	72.1	75.6±1.1	18.8
Nonlitter mates	418	3.16	74.4	76.1	286	3.14	77.3	78.7	704	3.15	75.6	77.1±0.5	20.4

[a] Owing to the important effect of size of litter on birth weight, an average birth weight adjusted to an average size of litter of three is given as well as the actual figures.

Corrected or not corrected, there is not much difference between the average weights of litter mates and nonlitter mates, although the former are slightly lighter according to both (corrected, litter mates 75.6±1.1 gm. nonlitter mates 77.1±0.5). Here, again, we have a slight indication that unfavorable conditions cause otocephaly, but in this case the difference is statistically of no significance.

The otocephali themselves are about 11 per cent lighter in weight than their normal litter mates. This difference, however, is to a large extent accounted for by their small heads. Taken as a class they show no evidence of malnutrition.

The direct search for indications of an environmental factor in determining otocephaly has led to rather meager results, although all lines of evidence agree in indicating that unfavorable environmental conditions have some influence.

There is one consideration which might well have discouraged such a search from the first. If otocephaly is determined by external factors of such nature as to act on litter mates alike, we should expect to find in many cases more than one otocephalus in a litter. There have been only six such cases and three of these are among descendants of the line from mating 13–19–1, in which the high frequency of otocephali (21.5 per cent) makes the chance occurrence of two in a litter a frequent probability. Of these six cases two were litters of two, both otocephali, while the others were in litters of three, two otocephali and one normal. Expressed in another way, otocephali have had 12 otocephalic litter mates (each pair counted twice) in a total of 152 litter mates, or 7.9 per cent otocephalic. They have had 64 otocephalic brothers and sisters among 1,107 in other litters, or 5.6 per cent. There is thus no appreciable tendency for otocephali to occur in the same litters, and, as we have previously seen, very little tendency for them to occur in the same matings, unless the whole line to which they belong is characterized by producing a high percentage. All environmental factors which act alike on litter mates, through effect on the condition of the dam or otherwise, are at once ruled out as factors of more than very secondary importance. The case is parallel to that of the piebald pattern in guinea pigs, which is determined nearly 60 per cent by nongenetic factors, but to no appreciable extent by factors which act on litter mates alike (*14*).

In looking for nongenetic factors peculiar to individuals of a litter, acting very early in development and not affecting to an important extent the final growth of the body, we are led at once to a factor which Mall [5] considered important, a delayed or temporarily faulty implantation. Since neither the condition of the dam nor the heredity of the young (within the line) is of prime importance, we must attribute such errors in implantation largely to chance. We must assume that normal relations are later established, but only after irreparable injury has been done to the most sensitive region in the developing embryo, which, according to Child's gradient hypothesis, should be at the anterior end of the central nervous system.

The minor grades of defect (grades 1 to 4) in which all the abnormalities seem to center around reduction of Meckel's cartilage (and to a less extent the hyoid) at first sight seem at variance with this theory, since Meckel's cartilage would hardly seem a likely location for the highest and most sensitive point in the gradient pattern. It has been demonstrated, however, that the branchial cartilages are produced from mesectoderm cells which wander down from the neural crest (*6*, *10*) (Miss Platt, 1897; Stone, 1922). A temporary arrest of development of the anterior end of the medullary plate might well cause a disturbance in the neural crest region, with consequences visible in the branchial cartilages even though not obvious in the brain. The arrest of the frontonasal process indicated in grade 5 leads to the series of stages (grades 6 to 12) in which the increasing arrest of the brain, beginning with the forebrain, is obviously the primary morphological factor.

GENERAL CONSIDERATIONS

Most work in genetics has necessarily dealt with variations of a rather superficial character whose relation to the great stream of heredity which determines the characteristics of the phylum, class, down even to the species, is like that of the ripples on the surface of a great river. One's impression on seeing a cyclopean in a litter of normal guinea pigs is that here is a variation of a more fundamental character, one which alters the entire course of development. At first thought it is somewhat disconcerting to this idea to find that the action of the hereditary factors in this case is so general in character that it is duplicated by that produced by a host of other agents, as cold, magnesium salts, butyric acid, mechanical destruction, lack of oxygen, in short anything which arrests development. The only thing that seems to be specific is the moment in development in which the agent is in action. The genetic factors which render individuals of Family 13 especially likely to follow the otocephalic mode of development appear, then, to be factors which alter the metabolic activities of the embryo at a critical time in development causing it to be unusually susceptible to inhibiting agents, among which the consequences of chance irregularities in implantation appear to be most important. That these genetic factors themselves do not tend to determine faulty implantation is indicated by the failure of Family 13 to

[5] Mall (*4*, *5*), however, considered cyclopia as wholly nongenetic. He drew a rather sharp line between a class of anomalies such as polydactyly, which he considered as wholly germinal, and real monsters, including cyclopia, clubfoot, anencephaly, spina bifida, etc., which he considered as wholly pathological. Wilder (*13*) also drew a line between germinal and pathological monsters, but a different line. He included cyclopia among the orderly symmetrical beings, "cosmobia," which he considered as germinal. The present data indicate that no sharp line can be drawn. Both germinal and environmental conditions play a part in determining cyclopia, and the same is undoubtedly true of other anomalies, including polydactyly and even variations in the piebald and tortoise-shell-color patterns in guinea pigs.

produce another type of monster—the partially double-headed kind—eight of which have been found in other stocks and which the experiments of Stockard (*8*, *9*) indicate are determined by arrests in development at a moment preceding the arrests which determine the cyclopean condition. There is no apparent correlation between the distribution of otocephali and other types of monsters among the experiments.

It would seem likely that genetic factors with an effect on general metabolism are very common, but at first thought it may seem as if such factors could play only a minor part in determining the type of development. But this is not a necessary conclusion. Study of the numerous orderly, nicely adjusted anatomical changes brought about in otocephali by a factor of this kind suggests the possibility that the whole course of development may be controlled by such factors. The fundamental properties of cells are much the same throughout the organic world, however diverse the structural patterns of the developed organisms. The Mendelian unit factors must be self-perpetuating entities within the cells. It may be possible that the action of Mendelian factors is merely to depress or accelerate metabolism. Their specificity lies in the unlocking of their activity at a particular moment when the cell has reached a particular condition. The pattern of development is then controlled by the particular succession of inhibitions and accelerations to which the various cells are subjected, as the result ultimately of the reaction of genetic factors with environmental ones. Just as the whole range of thought can be expressed by particular successions of dots and dashes in the Morse code, so the whole range of developmental patterns, from the one-celled alga to the sunflower, from the amoeba to man, may be the result of different sequences of inhibitions and accelerations. Development in a given cell lineage under this view is a chain reaction in which each gene reacts only in the presence of certain conditions, in part environmental relative to the cell lineage in question, and in part the result of the action of genes previously called into action.

This of course is taking an extreme view. It is not necessary to suppose that the action of all genes on cell metabolism is equally general. In addition to factors with a general influence released by a specific set of conditions, we may have genes which catalyze only a particular reaction and which can come into action only when the reacting substances are present.

On the discovery of units of heredity it was natural to compare them with the living units which had entered into previous speculations. The gemmules of Darwin, the biophores of Weismann, and all of their kind were essentially more or less sublimated representatives of the various morphological features of the adult organism. The developmental process was conceived of as a sorting out of these elements. This view, as Child has justly insisted (*1*), really explains nothing in development and is vitalistic in its implications. It was, nevertheless, adopted by many geneticists who began to look for determiners for the various parts of the body. Discoveries in genetics, however, while demonstrating ever more securely that the heredity of an organism is composed of constant units, have continually led away from this naïve conception of their nature.

In the case of otocephaly, specific unit factors have not been demonstrated, but their presence is probably indicated by the evidences of segregation in early generations and the sudden jumps in percentage of monsters at points in particular inbred lines. There is at any rate an

important genetic basis. Further, the case illustrates how a fundamental change in the developmental pattern at first sight perhaps suggesting the loss of determinants for parts of the head, may be explained much more satisfactorily as due to genetic factors with a very simple physiological effect, acting in conjunction with environmental conditions.

SUMMARY

Among about 40,000 guinea pigs recorded in genetical experiments of the Bureau of Animal Industry 82 monsters of the otocephalic type, or about 0.2 per cent, have appeared. These are classified in a practically linear series of 12 grades of defectiveness. The defects in the lower grades (1 to 4) center about arrest of Meckel's cartilage. Arrest of the fronto-nasal process seems responsible for grade 5. From grade 6 to grade 12 the primary feature is the progressive arrest of the brain. Grades 7 to 9 are cyclopeans. Grades 11 and 12 are almost headless.

The majority (50) have appeared in one inbred family, No. 13, in which the frequency is 1.5 per cent. There are marked differences in the percentages in different sublines of this family, there being indications of segregations of different tendencies in the early generations.

At two or three points in the pedigree there has been a sudden jump in the tendency. A line producing about 1 per cent jumped to 4 per cent in one of its sublines in the thirteenth generation. This gave rise to a branch in the nineteenth generation, which is producing over 20 per cent.

These observations demonstrate the importance of genetic differences between inbred lines. Analysis indicates, however, that there can be few or no genetic differences within such lines between the normals and the monsters. Their occurrence can not be explained as due to Mendelian segregation or to mutation, except where one whole subline becomes differentiated from its parent line in frequency of production.

Inbreeding merely brings to light genetic differences. Family 13 produced most of its otocephali in the later generations. All other inbred families, several with histories closely parallel to Family 13, produced the greater number in the early generations.

The genetic basis determines an individual, not a maternal, character. Females of Family 13 do not produce otocephali in outcrosses to the extent that they do in matings with brothers. Otocephali are more likely to appear in crosses in which both sire and dam have blood of Family 13 than in other crosses. Females are twice as likely to suffer the defect as males.

There is no appreciable difference in frequency in first and later litters or in last litters.

The seasonal distribution shows a slight predominance in the winter and early spring, when conditions are apt to be poor, agreeing with the fluctuations in size of litter and mortality of the normal young before weaning, with a not unexpected anticipation by half a month in the former case and a lag of a month in the latter.

Otocephali are born in slightly smaller litters than normals from the same matings. A slightly larger percentage of their litter mates die at birth and between birth and weaning than normals in other litters from these matings. There is a corresponding but statistically insignificant difference in birth weight between litter mates and nonlitter mates. Otocephali themselves are only slightly under weight and typically have a well-nourished, healthy appearance.

A litter mate of an otocephalus is not appreciably more likely to suffer the defect than a nonlitter mate.

It is concluded that the condition of the dam and external factors which act alike on litter mates through their effect on the dam play a part in determining otocephaly, but only a small part.

By elimination it is concluded that the main factor is probably chance delay or other irregularity in implantation acting on a genetic basis of susceptibility at a particular, critical moment in ontogeny to the resulting temporary arrest in development.

The grades of defect can be interpreted in harmony with Child's gradient theory.

The possibility is pointed out that many if not all genetic factors may have a simple accelerating or inhibitory effect on metabolism, as general in nature as those which seem to form the genetic basis of otocephaly, and that their control of the developmental pattern may rest merely on the order in which they come into activity as determined by conditions due to genes which have already acted.

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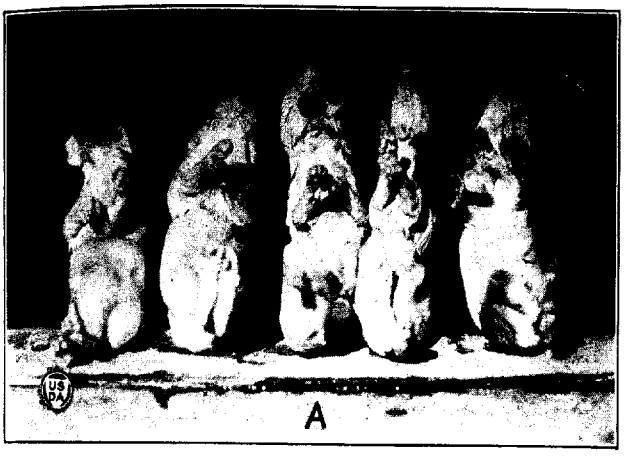
PLATE 1

A.—Otocephalic guinea pigs of grades 10, 9, 7, and 4 in comparison with a normal (extreme right). Ventral view.
B.—Lateral view of the otocephalic guinea pigs (grades 10, 9, 7, and 4, and the normal) shown in A.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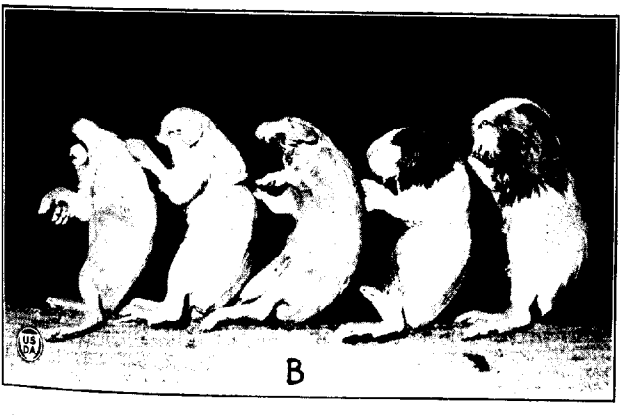
B

A METHOD OF AUTOMATIC CONTROL OF LOW TEMPERATURES EMPLOYED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE[1]

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In many scientific investigations the automatic control of low temperatures between narrow limits is very essential. Accurate automatic control of high temperatures is comparatively simple with electricity employed as the heating medium, but close control of temperature below that of the surrounding air is attended with more or less difficulty, for the reason that some form of refrigerating machine is necessary for removing the heat and it is difficult to compensate for the inherent lag present in machines of this character, and also in the thermostatic elements which are necessary for turning on and shutting off the supply of the cooling medium. In addition to the inherent lag of the refrigerating machine and thermal regulator, in certain work, as in studying the effect of low temperatures on plant life, where it is necessary that the plants be exposed to the sunlight, the heat from the sun adds greatly to the difficulty of accurately controlling the temperature. Furthermore, this difficulty is greatly augmented by passing clouds which suddenly shut off or let on this source of heat. The heat from the sun is about 7 B. t. u. per square foot per minute at the upper limit of the atmosphere. A part of this heat, however, is absorbed by water vapor, dust, etc., contained in the atmosphere; consequently, about 5 B. t. u. per square foot per minute is delivered on the earth's surface in the vicinity of Washington, D. C. Passing clouds reduce this supply of heat to perhaps 2½ B. t. u. per square foot per minute. It is obvious, therefore, that the problem of maintaining an approximately constant temperature is a difficult one and requires great care in order to secure satisfactory results. In order to minimize the thermostatic lag the instrument should have as small heat capacity as possible, so that it may respond quickly to slight changes in temperature. To procure a thermostat suitable for this particular work it was necessary to design one. In designing the instrument the object was to produce one that would show the same lag effect as the standard measuring instrument, the Beckmann thermometer. The instrument was designed by Dr. R. B. Harvey, and a description of it appeared in the Journal of Biological Chemistry, in January, 1920.[2]

Where the problem is one of accurately controlling the temperature only, it is an easy matter to cool the air by refrigerating well below the desired point and then heat back by electricity. By controlling the electric current the temperature may easily be maintained practically constant. In most cases, however, the relative humidity of the air is as important as the temperature, and should the temperature of the air be reduced below the dew point a part of its moisture will be deposited on

[1] Accepted for publication Aug. 3, 1923.

[2] HARVEY, R. B. A THERMOREGULATOR WITH THE CHARACTERISTICS OF THE BECKMANN THERMOMETER. In Jour. Biol. Chem., v. 41, p. 9-10, pl. 1. 1920.

the refrigerating coils, and when the air is heated back a low relative humidity will result. In many branches of work a low relative humidity is undesirable for the reason that it tends to desiccate the specimens.

In order to lower the temperature inside a chamber it is necessary to remove the heat. The quantity of heat to be removed from a chamber depends upon the difference in temperature between the inside and the outside of the room, upon the rate at which the heat passes through the surfaces of the room, and upon the quantity of heat produced or absorbed by the contents of the room. It is obvious, therefore, that in order to reduce the quantity of heat that must be removed, and hence the work required to remove it, adequate insulation should be provided. The better the insulation the more perfect the control of temperature, for the reason that the change in temperature is not so rapid when the refrigeration is discontinued, and therefore the quantity of heat that must be removed in order to establish constant temperature conditions is diminished. Furthermore, adequate insulation tends to maintain a constant temperature, and the rise in temperature, with the refrigeration entirely cut off, is slow, so that it becomes possible in some cases for the refrigerating plant to be shut down for several hours with only a few degrees' rise in temperature. This is important for the reason that, should the temperature control equipment break down for any reason, some time could be allowed for repairs, under average conditions, with a temperature rise that would not materially affect the experiments.

The experimental plant, as originally constructed, was to be used for maintaining the temperature of the culture room at 18° C. (64.4° F.). This part of the control was employed in work on the absorption of mineral nutrients by crop plants, using the conductivity method for determining the daily salt concentration in water cultures. A temperature accurately controlled to ±0.1° C. was convenient for the reason that it did away with temperature corrections in calculating the salt concentrations, and also made results in consecutive series comparable. With this equipment it was possible to maintain a temperature of 18° C. ±0.1° for long periods of time. In fact, interruptions were caused only by the discontinuance of the electric power, or breaks in the control system, and not because the apparatus itself failed to function.

The original installation, however, has now been extended for purposes to be mentioned later. The plant is divided into two parts, one for indoor and one for greenhouse work, each of which will be considered separately.

INDOOR COMPARTMENTS

The arrangement and construction of the indoor plant is shown in figure 1. The walls, floors, and ceilings are well insulated with cork board, and finished inside with a half-inch coat of hydraulic-cement plaster, so that the rooms can be washed out thoroughly and disinfected when necessary. There are three rooms in all—namely, a coil room, a culture room, and an instrument room, as marked on the plan.

The coil room is intended primarily as a "reservoir of refrigeration" from which cold air is drawn to maintain a constant temperature in the other two rooms. The temperature in the coil room is thermostatically controlled, the thermostat operating to stop and start the refrigerating machine on a temperature variation of about 3° F.

The temperature in the culture room and in the instrument room is maintained practically constant by drawing cold air from the coil room

and discharging it into the two other rooms, the quantity of cold air required to maintain the desired temperature being regulated by dampers in the discharge pipe from the fan. The dampers are manipulated by means of solenoids connected by bell cranks to the dampers, the solenoids being energized by electric currents controlled by thermostats located in these rooms.

In the culture room it has been found practicable to maintain a temperature for long periods with a variation in the liquid of not more than $\pm 0.1°$ C. from the desired temperature. The variation in the room, however, is somewhat greater, but not more than 1° C.

In the instrument room the temperature variation is slightly greater than in the culture room, owing to the opening of doors and to the presence of the person reading the instruments. A slight variation in temperature

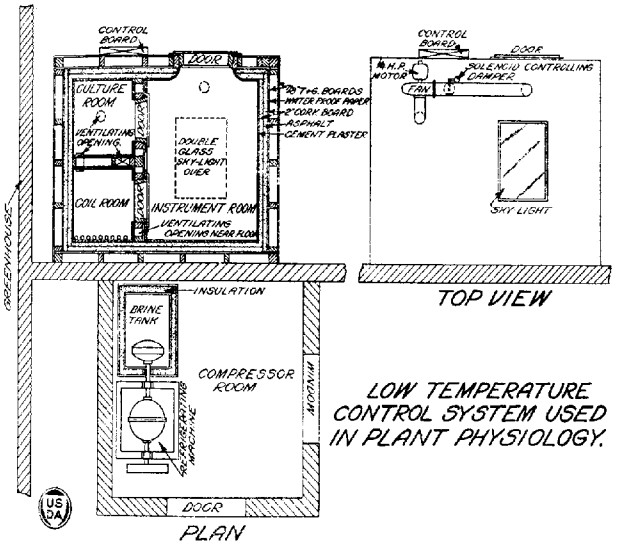


Fig. 1.—Arrangement and construction of compartments used with low-temperature-control system in plant physiology.

in this room, however, is of little importance, for the reason that the specimens are immersed in a solution and the short time in which they are kept in the instrument room has but little effect on the temperature of the liquid.

The original refrigerating plant consisted of two complete ¼-ton ammonia refrigerating outfits, one being held in reserve in case of a breakdown of the other. Direct expansion of the ammonia in the coils located in the coil room was employed. These machines were used because they were already on hand, but although great precautions were taken to prevent leaks, ammonia would escape from time to time and destroy or injure the plants and interfere with the work; subsequently a hermetically sealed sulphur dioxid outfit was installed and calcium chlorid brine was employed as the cooling medium, thus eliminating the danger of escaping ammonia. The temperature of the brine in an insu-

lated brine tank is controlled by a thermostat immersed in the brine and acting to start and stop the refrigerating machine on a temperature variation of approximately 3° F. The electric motor operating the brine-circulating pump is also started and stopped automatically by a thermostat located in the coil room. By this means the temperature in the coil room is held within a variation of 3° F.

WIRING DIAGRAM

A complete wiring diagram of the temperature-control system for the indoor portion of the indoor plant is illustrated in figure 2. As originally installed, no heating coils or fans were provided in the different rooms;

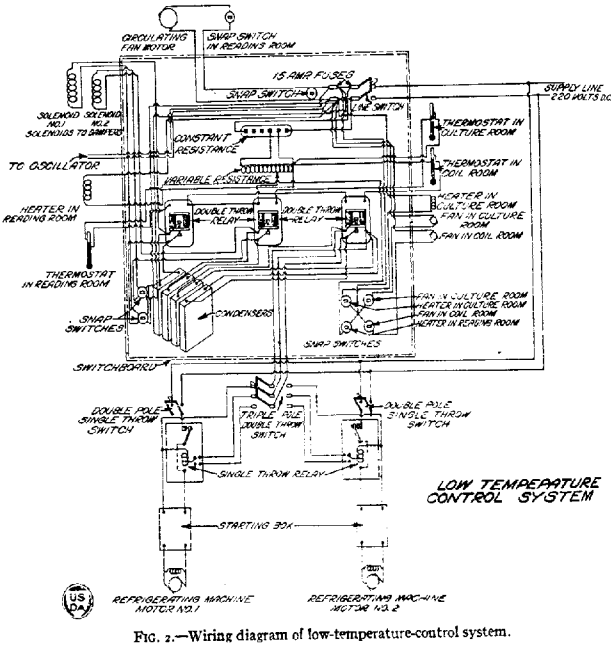


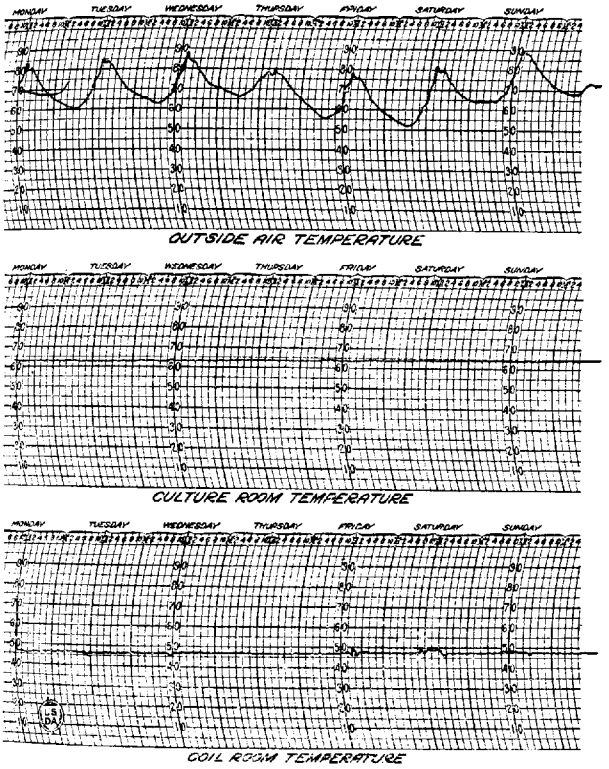
FIG. 2.—Wiring diagram of low-temperature-control system.

subsequently these auxiliaries were installed in an attempt to get a closer temperature control. Ordinary desk fans were placed in the rooms for the purpose of stirring up the air. The heating coils were employed for heating back to the desired temperature; that is, the thermostat controlling the flow of the cooling medium was adjusted to give a temperature slightly below that desired and the heating coils operated to bring the temperature up to the desired point. Very little, however, was gained by the employment of these auxiliaries.

TYPICAL TEMPERATURE CHARTS

Typical temperature charts are illustrated in figure 3. The upper chart shows the variation in temperature of the outside air covering a period of one week, while the middle chart shows the temperature main-

ined in the culture room during this time. The lower chart of the group ows the temperature maintained in the coil room during the same eriod. Check readings of the temperature in the culture room were also aken from time to time by means of a high-grade mercurial thermometer. ttention is called to the fact that the middle chart shows the tempera- ure of the air in the culture room, but since the cultures were propa-



IG. 3.—Typical temperature charts from indoor compartments. The apparent gradual rise of tempera- ture in culture room is due to the blank form having been slightly inclined when placed around the cylinder.

ated in water, the variations in temperature of the water were much ss than those of the air in the room.

The temperature of the outside air varied between 52 and 90° F., hile the temperature of the liquid in which the plants were propagated aried between the limits of ±0.18° F. (0.1° C.).

This portion of the plant was originally designed for maintaining a ractically constant temperature at or about 18° C. (±64.4° F.). Experi- ents have been conducted, however, at different temperatures between 8° C. and the freezing point, and the equipment has given satisfactory sults at any adjustment between these points. Closer control could ave been obtained at the lower temperature had the plant been designed r operation at the lower temperature.

GREENHOUSE COMPARTMENTS

The installation has been enlarged so as to provide four chambers located in one of the greenhouses. These chambers are used for growing plants in sunlight, partly to measure salt absorption in the light, and partly to study the effect of low temperature on the hardening of plants. The close control of temperature in these chambers is, as before stated, attended with considerable difficulty, caused by sunlight and the passing of clouds which suddenly shut off or let on this source of heat. Fairly accurate control of the temperature, however, has been obtained by reducing the temperature somewhat below that desired and heating back by an electrically heated coil. There is the great danger, however, in the heating-back method, of desiccating the plants too much, owing to low relative humidity. This has been corrected somewhat by providing a cold-air bunker at the bottom of each chamber. Cold air from this bunker is mixed with the warm air of the chamber by means of a fan and air valve. With this arrangement, entirely satisfactory control is not obtainable during the summer months, owing to the high temperature of the greenhouse, the large amount of solar heat, and the consequent high rate of air changes in the chamber.

It is believed that it is impracticable to accomplish very accurate temperature and humidity control during the summer months if attempted on a small scale. Accurate control can perhaps be economically obtained only when the equipment is large enough to provide for the necessary heat interchanges and for the control of humidity by a spray system.

Typical temperature charts are illustrated in figure 4. The upper chart shows the variation in the greenhouse temperature, and the lower chart shows the temperatures maintained in one of the cold chambers during the same period.

Referring to the lower chart, the numbered points are explained as follows:

1. Shows the temperature in the chamber at the beginning of the experiment. In about one and one-half hours the temperature of the chamber was down to the desired point and the control equipment began to function. A temperature of about 23° F. was maintained for several hours to insure the satisfactory operation of the control equipment and the cooling of the chamber.
2. Plants placed in the chamber.
3. Plants placed in and removed from chamber (2 lots).
4. Plants placed in and removed from chamber and regulator reset to maintain a slightly lower temperature.
5. Plants placed, then box opened and plants allowed to thaw before removing.
6. Repetition of No. 5.
7. Plants placed, temperature regulator reset.
8. Plants placed (2 lots), temperature regulator reset.
9. Plants placed and removed.
10. To this point the temperature was controlled by heating back with electricity. After this point the temperature was controlled by refrigeration alone, that is, by starting and stopping the refrigerating machine automatically.
11. Experiment finished and compressor stopped.

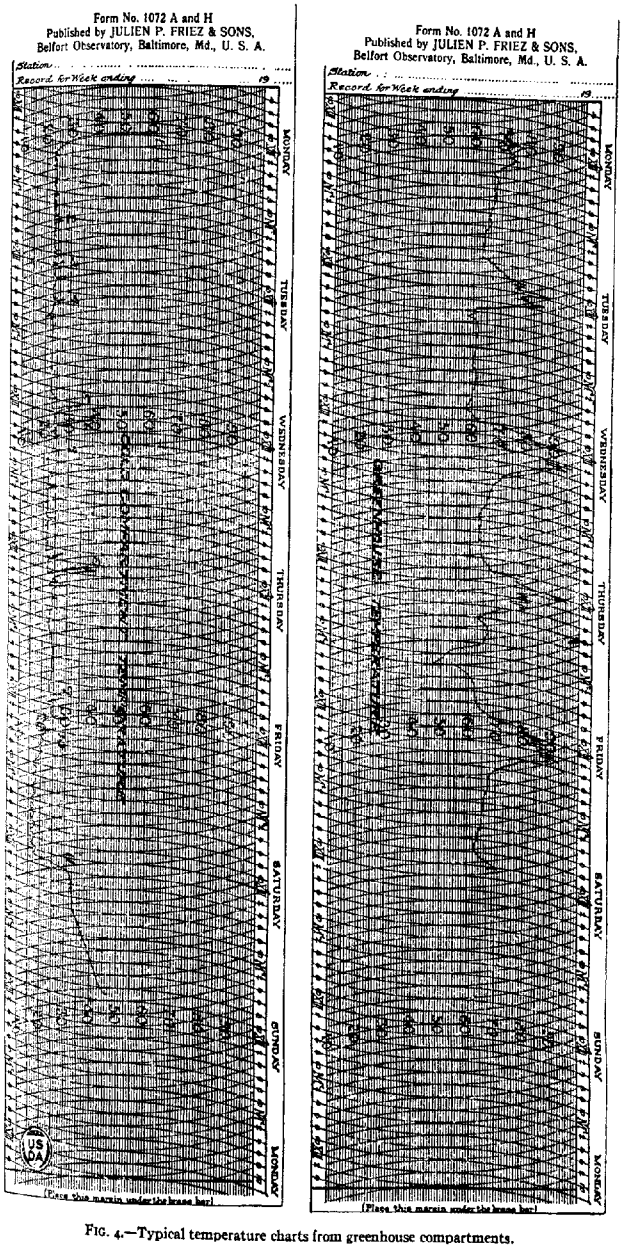


FIG. 4.—Typical temperature charts from greenhouse compartments.

It will be noted, by referring to the chart taken in the compartment, that between the points marked the temperature was fairly constant for a given setting of the thermal regulator; also, that the portion to point 10, when considered with that between 10 and 11, shows very distinctly the effect of heating back with electricity. The portion between 10 and 11 shows with equal clearness the degree of control obtainable with this equipment when the control is effected by starting and stopping the brine pump automatically by means of thermostat located in the cold chamber.

TYPE OF REFRIGERATING MACHINES

In the production of low temperatures for experimental work in connection with growing plants, the medium employed in the refrigerating plant should be carefully considered. In most types of refrigerating machines leaks of the refrigerant from the various joints in the system are likely to occur sooner or later and should ammonia be the refrigerant the results would undoubtedly prove disastrous to the growing plants. Carbon dioxid, on the other hand, would probably accelerate their growth to an extent that might interfere with the experiments. The refrigerating machinery, therefore, should be kept entirely away from the growing plants, unless the apparatus is of a type in which the refrigerating medium is hermetically sealed within the machine. In any low-temperature work where it is necessary or desirable to evaporate the refrigerating medium directly in the cold chambers, great care should be exercised to prevent leaks in the pipe coils. The pipe coils preferably should be continuous, that is, without joints. In case joints are necessary they should be carefully made and then soldered.

Unless very low temperatures are desired, the brine-circulating system offers, perhaps, the best solution. With this system the brine may be reduced to a low temperature in an insulated tank by evaporating the refrigerating medium in coils which are immersed in the brine; and the low-temperature brine in turn may be circulated through coils located in the cold chambers. The advantages of this system are: There is little danger from leaks from the brine coils; the temperature in the cold chambers may be controlled between closer limits; it is possible to store up a considerable amount of refrigeration by having a large volume of brine which is available in case of a temporary breakdown of the refrigerating plant.

The disadvantages of a brine system are: A greater initial cost, due to having to install both direct-expansion coils in the brine tank, and brine coils leading to the cold chambers and in the chambers themselves; a pump for circulating the brine through the coils and the additional cost of operating the pump; the cost of additional power due to having to operate the refrigerating machine at a lower back pressure in order to compensate for the double heat transfer from the air in the cold chambers to the brine and from the brine to the evaporating refrigerant.

EXCRETIONS FROM LEAVES AS A FACTOR IN ARSENICAL INJURY TO PLANTS[1]

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INTRODUCTION

Calcium arsenate has been applied as a dust for the control of the cotton-boll weevil for several years, with such successful results that its use is increasing rapidly, about 16,000,000 pounds having been used during the season of 1922. Irregularities in the physical and chemical properties of commercial brands and plant injury by material which conformed to the specifications of the Bureau of Entomology, United States Department of Agriculture, led to a physical and chemical study of this arsenical. The progress made on one phase of the problem during the summer of 1922 is reported in this paper.

CAUSES OF ARSENICAL INJURY

The compounds of arsenic which are soluble in water injure the plants to which they are applied with varying degrees of seriousness. Holes may appear in the leaves, partial defoliation may occur, or the entire plant may be killed, depending upon the concentration of the solution used and the susceptibility of the plant treated. The compounds of arsenic that are only slightly soluble in water, however, are much less toxic—even nontoxic—to plant life. They are therefore very important in the control of insect pests.

In general, the toxicity of an arsenical to plants depends largely on the percentage of "water-soluble arsenic" it contains; that is, the percentage of arsenic which will enter solution under certain prescribed conditions.[3] This soluble arsenic may have its origin in impurities in the material, actual solubility of the material, or hydrolysis of the material by water.

However, this is not a complete explanation of arsenical injury. Plants differ in susceptibility to arsenic. For instance, potato vines tolerate Paris green, with its relatively high soluble arsenic content, whereas bean plants can not be safely sprayed with any arsenical.

Weather conditions constitute a third variable factor. Erratic results, believed to be due to the effect of temperature and humidity, sometimes

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[1] Accepted for publication Aug. 11, 1923. This paper was read at the meeting of the American Chemical Society at New Haven, Conn., Apr. 5, 1923.

[2] In carrying out this work the author was assisted by S. B. Hendricks, Entomological Field Assistant, Delta Laboratory, Bureau of Entomology, United States Department of Agriculture.

[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. As compiled by the Committee on Revision of Methods. Revised to Nov. 1, 1919. 417 p., 18 fig. Washington, D. C. 1920. Bibliographies at ends of chapters.

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follow standard procedures. Fernald and Bourne,[4] after observations of lead arsenate spraying extending over a period of 12 years, state that the injury to such tender foliage as that of peach and plum is influenced by both temperature and humidity. They have defined the limits of the temperature-humidity relation within which it is safe, and beyond which it is unsafe, to use lead arsenate on such trees.

Certain chemical factors also are of importance, particularly the quantity and character of the salts in the water with which a material is applied. The reaction of these salts with the arsenical may liberate large quantities of soluble arsenic.[5] Haywood and McDonnell [6] have pointed out the susceptibility of dilead arsenate to alkali carbonates and soluble chlorids, and McDonnell and Smith [7] have shown the nature of the reaction in the case of the chlorids. Their work suggests that the burning by dilead arsenate frequently observed on the Pacific coast, and hitherto attributed to hydrolysis by the heavy and recurring fogs of that region, is really due to the salt spray entrapped by these fogs.

Granting the possibility of decomposition of the arsenical by salts in the water used and by material deposited from the air, may the plant contribute to the injurious tendencies by furnishing other substances capable of causing decomposition? Patten and O'Meara,[8] recognizing this possibility, suggested that the carbon dioxid given off by the leaves might be a factor. Their experiments showed that calcium arsenate was very sensitive to aqueous carbon dioxid solutions, giving much more "soluble arsenic" than could be dissolved under similar conditions in pure water.

RELATION OF DEW TO ARSENICAL INJURY

The occasional burning which could not be traced to poor material, usually most pronounced when the dew was heaviest and drying rapidly in the morning, and observed during dusting experiments with calcium arsenate, led to an examination of dew on cotton leaves. Instead of an acid reaction, as would have been the case had the expected free carbon dioxid been present, the dew gave a reaction alkaline even to phenolphthalein, indicating the presence of soluble hydroxid or salts of very weak acids. In each of the many tests made in different localities the result was the same. The alkalinity seemed to be localized around the main ribs of the leaves. The soil in the cotton fields and that in the neighboring roads did not give an alkaline reaction, so that the effect could not have been due to dirt splashed up from the ground by the rain or blown on by the wind. Dew from many other plants, including corn, china berry, grass, cocklebur, and several other weeds in the same fields was likewise tested. In no case was the reaction alkaline. It would therefore appear that the condition is natural and perhaps peculiar to the cotton plant, at least in the region around Tallulah, La., where these observations were made.

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[4] FERNALD, H. T., and BOURNE, A. I. INJURY TO FOLIAGE BY ARSENICAL SPRAYS. I. The lead arsenates. Mass. Agr. Exp. Sta. Bul. 207, 19 p., 23 fig. 1922.

[5] ONG, E. R. de. THE RELATION OF HARD AND ALKALINE WATERS TO THE PREPARATION AND DILUTION OF SPRAYS AND DIPS. *In* Jour. Econ. Ent., v. 15, p. 339–345. 1922.

[6] HAYWOOD, J. K., and MCDONNELL, C. C. LEAD ARSENATE. U. S. Dept. Agr. Bur. Chem. Bul. 131, p. 46. 1910.

[7] MCDONNELL, C. C., and SMITH, C. M. THE PREPARATION AND PROPERTIES OF LEAD-CHLOR ARSENATE, ARTIFICIAL MIMETITE. *In* Amer. Jour. Sci., v. 42, p. 139–145, 2 fig. 1916.

[8] PATTEN, Andrew J., and O'MEARA, P. THE PROBABLE CAUSE OF INJURY REPORTED FROM THE USE OF CALCIUM AND MAGNESIUM ARSENATES. *In* Mich. Agr. Exp. Sta. Quart. Bul., v. 2, p. 83–84. 1919.

About 1,300 cc. of dew obtained from mature cotton plants was collected. Duplicate analyses on 500 cc. portions gave the following sults, expressed as parts per million:

Total solids	1,023
Silica (SiO_2)	13
Oxids of iron and aluminum (R_2O_3)	17
Sulphur trioxid (SO_3)	26
Chlorin (Cl)	19
Calcium oxid (CaO)	529
Magnesium oxid (MgO)	100
Carbon dioxid (CO_2) (by titration)	618

A small proportion of carbon dioxid (equivalent to 40 parts per million calcium carbonate) was present as carbonate, all the rest being in the rm of bicarbonate. No attempt was made to determine the alkali etals, but the agreement between the substances determined and the tal solids indicates the presence of but little such material.

Evidently the principal constituents are bicarbonates of calcium and agnesium. Whether these have come into the dew by osmosis or by tual exudation was not determined, the former being more probable.

Hardness titrations were run on several other small collections of dew. ll showed large quantities of bicarbonate and relatively small quantities carbonate. The only other determination of total solids gave 960 rts per million, which agrees closely with the results of the analysis of e first collection. This dew was used for a soluble arsenic determinaon on a sample of calcium arsenate, showing with boiled distilled water 8 per cent of soluble arsenic oxid. With the same dilutions and nditions, 8.7 per cent of arsenic oxid was dissolved by the dew.

A properly made calcium arsenate, then, may undergo extensive decomsition after being applied to a plant. Of course, in interpreting the sults of the laboratory experiments it must be remembered that the lative proportions of dust and liquid, as well as their degree of agitaon, may largely influence the result. As the proportion of dust and uid is a prime factor in determining the percentage of arsenic dissolved om calcium arsenate, an attempt was made to estimate it under field nditions. Freshly dusted plants were analyzed to determine the antity of arsenic held by them, and the quantity of dew present was timated. A normal dusting with a hand gun left about 0.4 gm. of lcium arsenate upon each plant used in the tests, and a dripping dew t between 100 and 200 cc. of moisture on each plant. This is equivait to a concentration of from 2 to 4 gm. of arsenate per liter, as compared th 2 gm. per liter, used in the water-soluble arsenic determinations. there must be an enormous variation in this concentration, these figures e merely approximate.

The calcium arsenate used for the soluble arsenic determination with w was part of a lot which had caused some damage to cotton at Rosele, Miss., earlier in the season. No other sample was taken for comrison, but numerous experiments were made, using tap water, which is very hard with calcium, magnesium, and ferrous bicarbonates, thus mewhat resembling the dew. Nine samples of calcium arsenate[9] ich averaged 0.10 per cent water-soluble arsenic oxid with distilled ter gave with tap water from 0.8 to 2.2 per cent, with an average of per cent of arsenic oxid. Eight samples from the stock which caused trouble at Rosedale, averaging 0.11 per cent with distilled water,

[9] Representing the products of four manufacturers.

gave with tap water from 7.8 to 10 per cent, with an average of 8.5 pe cent of arsenic oxid. Thus, samples of calcium arsenate which appeare practically identical, according to the two tests usually applied in th chemical control of calcium arsenate, actually differed markedly.

The explanation of this difference must be sought in the relativ quantity of some other constituent, or in the physical nature of th calcium arsenate, methods for investigating which are being developed The most plausible chemical explanation is that insufficient free lime i present. Since free lime keeps the water-soluble arsenic content low, i is possible that a certain minimum requirement to react with the con stitutents of the dew is necessary. A method for determining free lim in calcium arsenate is being studied at the present time, with a view t testing this point.

SUMMARY

Dew on Upland cotton plants contains large quantities of salts whic in laboratory tests so act upon calcium arsenate as to increase greatl the water-soluble arsenic content. That this is the cause of the erratic injury noted remains to be proved. What is true of the cotton plant may be true of other plants. Excretions from leaves, therefore, must be considered in explaining arsenical injury to plants. This factor may be of value in estimating the suitability of an arsenical for a particular purpose.

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